THE
AMERICAN NATURALIST

VOL. LXXXI *March–April, 1947* NO. 797

THE AMERICAN SOCIETY OF NATURALISTS

DUPLICATION OF CHROMOSOME PARTS AS A FACTOR IN EVOLUTION[1]

DR. C. W. METZ

DEPARTMENT OF ZOOLOGY, UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA

To me the duty of addressing you this evening here in Boston is a particularly disconcerting one. My mind goes back unavoidably to that corresponding occasion twenty-four years ago, when those of us whom the gods favored had the privilege of listening, here, to what marked one of the high spots in after-dinner speeches before this august society, especially from the standpoint of entertainment. If I quote the opening sentences you will recognize the speaker to whom I refer, and I think you will also readily see why I am disconcerted. He began like this:

"Our Society requires its retiring president to close the annual meeting with a discourse or sermon—a task which has become increasingly difficult, for every year the program of the morning and afternoon sessions becomes more abstruse and therefore makes greater demands on our attention and the lingering memories of past presidential rhetoric invite to more odious comparisons. To me the task was the more arduous, because I

[1] Address of the retiring president of the American Society of Naturalists, Boston, Massachusetts, December 30, 1946. The address was illustrated with lantern slide figures, only a few of which are included here. The original work reported in the last part of the paper was supported in part by the American Philosophical Society and the Rockefeller Foundation.

had been busy for many years in remote fields of entomology in which few of you are interested, and because it fell to me at an inopportune moment, while I was in the very act of laying—if you will pardon a French expression—a volume of some 1,100 pages on ants. This racking oviposition leaves me reduced to a mere blob of corpora lutea and so feeble that I can only crawl, in search of a text for my sermon, to the next Encyclopaedia Britannica article, which is not 'ant-eater,' but 'Antaeus.' "

The speaker, of course, was Harvard's William Morton Wheeler, here on his own home ground. His topic was "The Dry-rot of Our Academic Biology"[2]—a subject which delighted his soul, and which in his hands delighted his audience even more. At least it delighted all those who, like myself, were around the tender age of thirty and hence could get huge enjoyment out of the squirms of our sedate elders as Wheeler's shafts went home. Then, as now, our society was supposed to be very dignified. The speaker's table was large and was on an elevated platform, so many of the elder statesmen—recipients of Wheeler's darts—flanked him on either side (like the gentlemen about me) in full view of the rest of us. I would like nothing better than to entertain you to-night with Wheeler's address. But custom forbids second-hand orations. A failure to cite any of his remarks, however, would be an equal injustice, particularly in view of the fact that we would thereby miss the opportunity to compare the pathological symptoms of a quarter of a century ago with those of to-day and also to glean some bits of philosophy which are pertinent to this evening's discussion. So let me recite a few short passages, mainly from the early part of his discourse.

Where we left off Wheeler was speaking of Antaeus. He went on to recount the exploits of Antaeus, who, as you know, was invincible as long as he was in contact with the earth. But "one day Hercules came along and, knowing the secret of the giant's strength, raised him aloft

[2] W. M. Wheeler, 1923. *Science*, 57: 61–71.

and strangled him in the air." Wheeler used this as an object lesson for biologists, saying that the "meaning of the myth is that even an agile and vigorous mortal had best keep his feet on the concrete if he wishes to avoid death at the hands of the Hercules of abstraction" and that "the myth remains to this day as one of the most beautiful expressions of the practical man's attitude toward those who place too much confidence in their more abstract intellectual operations."

From this he turns more specifically to biology professors. "After securing this text (he says) there was difficulty with the title of my sermon. I could not decide whether to call it the 'tommy-rot' or the 'dry-rot' of our academic biology. I finally chose the latter, because some of our activities so closely resemble the inroads of the fungus *Merulius lacrymans* in old timber. . . ." Then he referred to "the disappointing spectacle of our accomplishments as more or less decayed campus biologists" and to his opinion that "even if we concede that the damned professor (here he is quoting Bismarck) is an extraordinary being because he has sufficient inertia to specialize for a lifetime in a particular department of learning, we must admit that he will grow old like the most ordinary individual of his species. . . . At forty, if not sooner, his sense-organs, musculature, endocrines, emotions and memory will begin to atrophy and his intellectual processes will become more and more stereotyped, dogmatic and abstract. . . . he will become a creature increasingly infatuated with generalizations, relationships and hypothetical explanations, . . .

"Unfortunately we have no intelligence tests for individuals with a mentality of more than eighteen years, and biologists are supposed to be older, though some of them somehow manage to harmonize a physical age of forty to sixty with a mentality of eight to fourteen." However, "it is not from such professors that the Merulius spores proliferate most profusely, but from those who have a physical age of forty to sixty and a mental age of eighty to one hundred and five."

Perhaps these samples will serve to give you a portrait of the biology professor of Wheeler's day. You must be the judge of whether or not he has changed in the past quarter century.

And now, in turning to what is supposed to be my real topic, let me pick out just one more bouquet from Wheeler. This one could hardly have been improved if it had been aimed directly at us to-night. Speaking of the esoteric efforts of professors he says: "This type of senescent compensation is . . . clearly exhibited by old or prematurely old taxonomists, morphologists and geneticists, who derive from static fictions like species, unit characters, genes, etc., a certain feeling of potency, of having their fingers on the very vitals of organic reality."

With this last remark ringing in our ears should any one of us have the temerity to attack such a problem as I have chosen for this evening's discussion? Probably not. Probably by so doing, I admit membership in that numerous company of "more or less decayed campus biologists." But then, I am past forty and presumably beyond hope, so please bear with me. At least these quotations from Wheeler ought to convince you that I realize the nebulous nature of the ideas I may deal with when I get aloft and well out of contact with mother earth.

As has often been pointed out, evolution can not be explained on the basis of loss or simple alteration of materials already present in the germ-plasm. New elements must be added. Otherwise, we would have to assume that the "primordial amoeba" was endowed with all the germinal components now present throughout the wide range of its descendants, from protozoa to man. It is this problem of acquiring new germinal materials which concerns us. Observing Wheeler's admonition to keep our feet on the ground, at least for a few moments, let me begin by directing attention to some of the simpler and more obvious aspects of the subject.

The discussion will be confined to the chromosomes. Whether or not we agree that the chromosomes represent

the germ-plasm, we may be confident that they represent a major part of it—a part more than ample for present purposes. Our primary task, therefore, is to get some conception of how new materials have been added to the chromosomes during the course of evolution.

To enter effectively into the complex genic organization of a chromosome, a new unit would first have to be of the proper size; it would also have to be self-reproducing; it would have to be able to correlate its rate of growth and reproduction with those of the other genic units; it would presumably have to possess the peculiar ability to fit into the so-called "gene-string," attaching itself in the proper manner to its neighbor units on either side; and it would, of course, have to be able to function in harmony with its compatriots. Conceivably, the so-called "plasmagenes" now coming to light, especially in the studies on microorganisms, may prove to be such units. But at the moment it seems more probable that they have been derived from the nucleus than that they are potential contributors to the nuclear mechanism.

From the practical standpoint, therefore, we need only consider additions to the germinal material which come primarily from the chromosomes themselves. If we add to the chromosome complement of a nucleus whole chromosomes, or parts of chromosomes, we are adding something which already has the properties necessary for perpetuating itself.

Addition alone is not sufficient for evolutionary purposes, however, as has often been pointed out. We have not added anything new. Qualitative changes must ensue in the duplicated materials. We can not assume that present-day animals and plants have evolved from the "primordial amoeba" merely through the process of multiplying the relatively simple germinal constituents originally present in that primitive organism.

Were there time, it would be interesting to speculate on how much chemical resemblance there may be between to-day's genic "units" and the primordial ones. Suffice

it to say that to-day's genes, or many of them, are probably much more complex than their remote ancestors. Considering the variety of characteristics presumably common to all genes as we know them, it is not unreasonable to suppose that to-day's genes have all descended from one original gene. We may picture that gene as multiplying to give many replicas of itself. Subsequently, some of these underwent qualitative change, while others remained as they were and continued to perform their old functions. This process continued. Sooner or later chromosomes were formed and groups of germinal materials acted as units. Once embodied in chromosomes, the genes had to act in concert, laying the foundation for the present-day precision of chromosome behavior. For continued evolution, further additions of germinal material were required from time to time. Here duplication of chromosome parts came in as a major factor. If units A, B, C became duplicated in a chromosome, any one of them could undergo qualitative change while its counterpart kept on performing its old functions. With subsequent modifications and adjustments in function of other genes, the unchanged counterpart might itself become modified or lost without detrimental effects. Thus, ultimately, the original, simple germinal materials might no longer exist as such in the genetic mechanism. Presumably, repeated additions and qualitative modifications could transform all the germinal material in the course of time and give rise to any degree of complexity compatible with the proper functioning of the cell and the organism in the struggle for survival in nature.

In the latter part of this excursion our feet have admittedly soared well off the ground. But perhaps the flight has served to emphasize the two major aspects of our problem, first, the addition of chromosome materials, and second, their subsequent qualitative change. We are primarily interested in concrete evidence and in interpretations to be derived therefrom, but unfortunately, the

subject is one about which we know relatively little; hence the great temptation to speculate. My main reason for treating the topic at this time is its importance. There are additional reasons, however; one is the fact that a large part of our specific information in the field in question has been obtained within the past ten or twelve years and is not yet widely known. Another is that it is interesting at this stage to see what paths may be open for further progress.

Needless to say, it is impossible to give an adequate review of our subject in a discussion like the present one. We must treat mainly not clear-cut facts and demonstrated results, but preliminary evidence, indications, probabilities and possibilities, most of which would need full discussion for an adequate understanding. So, for the most part, I will have to confine myself to general aspects and interpretations, without attempting to elaborate experimental details. And I will have to omit all discussion of methods and mechanisms—such as the mechanisms by means of which chromosome rearrangements are brought about.

Simple doubling of the chromosome groups to produce tetraploidy would seem to be the ideal first step toward our goal of increasing the chromosome materials, and this process appears indeed to have performed an evolutionary function in some groups of plants. Nevertheless, the evidence seems clearly to show that polyploidy is not a prime factor in evolution generally, particularly among animals. Likewise, there are good observational grounds for ruling out the addition of single, whole chromosomes as a broad basic evolutionary process (presumably because of the deleterious effect of the unbalance thus created). This leaves us, then, with the particular subject of to-night's discussion: "The Duplication of Chromosome Parts." To my mind, the duplication of chromosome parts, together with its consequences, has probably been one of the most important factors in evolution, if not the most important. But we must admit that

the inference is arrived at by a process of elimination rather than from specific evidence.

Up to 1933 progress was very slow in the field in question. Comparative cytological studies on chromosomes of different species, and genetic evidence of translocation of chromosome parts (*e.g.*, that of Bridges in 1918) suggested that chromosomes are occasionally enlarged by the addition of pieces without compensating loss. But these studies furnished little precise information about what happened or how it happened. A similar suggestion came from the genetic evidence of so-called "duplicate genes"—that is, genes which appear to be duplicates of one another in their genetic effect, but are located in different chromosomes or different parts of one chromosome. Such cases are known in both animals and plants —and, of course, suggest an origin from duplication of chromosome parts.

An especially significant step along this line was made when Sturtevant showed in 1925 that the dominant mutant Bar-eye in Drosophila arose through duplication of something already present in the chromosome. Bar arose as a spontaneous mutation. Subsequently, other comparable cases have come to light in various organisms, especially among the mutations induced by irradiation.

It was not until the giant salivary gland chromosomes of the Diptera came into the picture in 1933, however, that the field became wide open for an accurate determination of what was taking place in such cases. Our fondest dreams could hardly create anything more nearly ideal for the purpose than these enormous chromosomes found in the larvae of Diptera. For any of you who may possibly be unfamiliar with them, let me indicate some of their peculiar advantages. Their size and general characteristics in comparison with their counterparts in "ordinary" cells are shown in the photomicrograph (Fig. 1). This figure is from one of the fungus flies, Sciara. The enormous size of these chromosomes

may be seen by comparing with the chromosome figure in the lower right-hand corner, which is a photomicrograph of the chromosome group in an ordinary mitotic cell of this same species taken at the same magnification. As you will see, the species has four pairs of chromosomes and each salivary gland chromosome represents a pair. In other words, what appears to be a single salivary gland chromosome is really a pair of homologs intimately

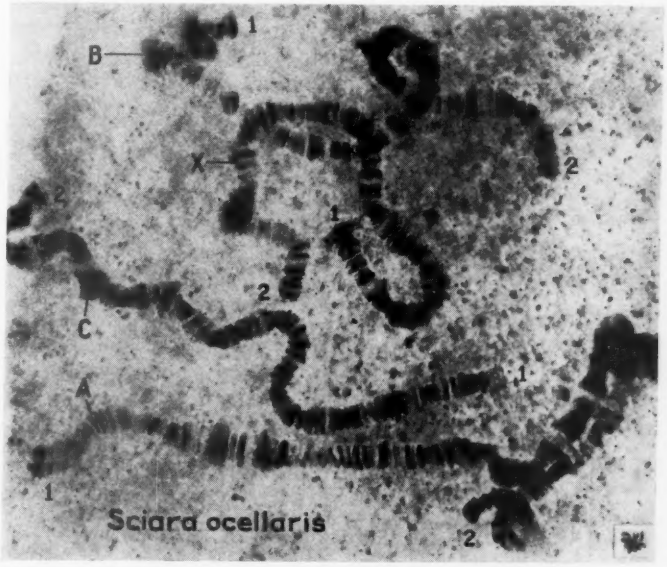

FIG. 1. Photomicrograph of salivary gland chromosome group of the fungus fly *Sciara ocellaris* Comstock, from an aceto-carmine smear preparation. ×575. See text for explanation.

united side by side so that each chromatic cross-band or disk in the one is joined to its mate in the other—the two together making a continuous band or disk across the more or less cylindrical "chromosome." These cross-bands or disks (they are actually disk-shaped) have individual characteristics of thickness, degree of staining, etc., which differentiate them from one another, with the result that not only each chromosome, but even any short

region within a chromosome, has its own distinctive pattern which distinguishes it from other regions. This pattern is highly constant and makes possible an accurate comparison of chromosome regions.

Needless to say, the Bar-eye mutant in Drosophila was one of the first subjects to be investigated with the aid of the giant chromosomes. The Bar flies were found by Bridges, and independently by Muller, Prokofyeva-Belgovskaya and Kossikov, to possess a duplicated piece of the normal chromosome, consisting of a segment contain-

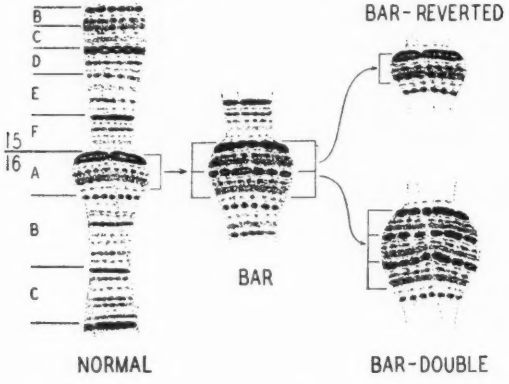

FIG. 2. The Bar duplication in *Drosophila melanogaster* as seen in the salivary gland chromosomes. (After Bridges, 1936, *Science, 83:* 210–211).

ing at least seven of the chromatic disks or cross bands. The condition in the normal chromosome is shown at the left in Fig. 2. The Bar segment is enclosed in the bracket. The condition in Bar is shown in the middle, where this segment is duplicated. These investigations also corroborated Sturtevant's earlier evidence that "Double-Bar" and other extreme forms were the result of further duplications of this same segment. The process of unequal crossing over which gives the triple condition from Bar may also cause a reversion back to the single condition, as shown at the right in Fig. 2. Another significant aspect, as shown by Sturtevant, is that the effect of the duplicated segments depends not only on

their number but on their spatial relations. In other words, we have here not only a duplication of parts, but what is known as a "position effect." For example, if three Bar segments are present in one homolog of the pair, and one is present in the other, the phenotypic effect is more extreme than that produced when two Bar segments are present in each homolog.

Studies on the salivary gland chromosomes also soon brought to light another line of evidence—this time based on normally occurring conditions in wild stocks. In an intensive study of the salivary gland chromosomes of *Drosophila melanogaster*, Bridges found cases of duplicated or "repeat" regions which are characteristic of the normal chromosomes of the species. Such regions are not only alike in their band patterns but tend to show their homology by uniting with one another side by side in somatic synapsis.

Some of these repeats are essentially similar to the Bar repeat except that the pattern in one segment is reversed and thus is the mirror image of that in the other segment. Such a repeat is an adjacent, reversed repeat, whereas Bar is an adjacent, serial repeat. Another type of repeat differs from these two in that the repeat segments are not adjacent but well separated. In such cases the repeat regions usually come together in somatic synapsis and when forcibly separated during preparation of the "smears," lateral strands of chromosome material connect homologous bands in the two segments (see, *e.g.*, Fig. 5, regions 33–34 and 38–39, Bridges, 1935, *Jour. Hered.*, vol. 26, p. 62; also Fig. 4 of present paper).

All the repeats considered up to this point consist of several bands, involving in each case a definite pattern. For convenience such repeats may be called "pattern repeats." Bridges immediately pointed out the possible evolutionary significance of these repeats. In the fungus fly Sciara we also found such conditions, to which I will refer again presently. Adjacent repeats are apparently more numerous than well-separated ones in wild popula-

tions—and for reasons that will appear later they are, so far as known, always "reversed repeats."

In addition to these "pattern repeats," which unquestionably represent duplications, there is another type of structure found commonly in salivary gland chromosomes which may also be derived by duplication. This is the so-called "doublet" originally identified by Bridges and interpreted as a repeat. A doublet involves only two bands—one band supposedly the duplicate of the other. An example of a doublet is shown at the upper left in Fig 3*a* (indicated by the dotted line). The particular reason for supposing the two bands to be duplicates is that they converge at the margin to form a lens-shaped structure, like two saucers placed face to face. The convergence is interpreted as evidence of synaptic attraction, which indicates homology. Our evidence from Sciara tends to corroborate Bridges' contention, as do also subsequent experimental studies on Drosophila. Some authors, however, interpret the doublets as single units.

This matter is important and merits more careful examination than we can give it here. The present state of the subject may perhaps be summarized as follows: Bridges has recorded at least three cases of irradiation experiments which he interprets as involving breakage of the chromosome between the two bands of a doublet, with consequent separation of the two—indicating that the doublet is really a double structure. We have found in wild populations of Sciara cases in which what appears to be a doublet in one chromosome of a pair is matched by a single band in the other homolog. An example is shown in Fig. 3. As you will see, the single band in one homolog resembles half of the doublet. All three possible conditions are found—that in which both homologs have the doublet structure and that in which both have the single band, as well as the heterozygous condition just referred to. Two conditions are shown in Fig. 3. In the chromosome at the left, at the locus indicated by the

dotted line, the doublet structure is present in both homologs. In the other chromosome the heterozygous condition is present. The interpretation of this figure is shown in the diagram. Both the appearance and behavior here suggest strongly that the doublet is the result of duplication.

Several genetic investigations in Drosophila point in the same direction. I will cite only Lewis's study of the

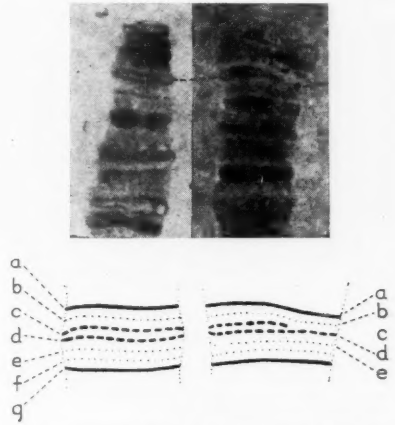

FIG. 3. *a*, photomicrograph, and *b*, explanatory diagram, illustrating the "doublet" condition in the salivary gland chromosomes of *Sciara ocellaris*. Chromosome at left shows homozygous doublet condition at dotted line. Chromosome at right shows heterozygous condition as indicated schematically in the diagram at the right. See text for description. Photomicrograph ×1500.

Star-asteroid locus in *Drosophila melanogaster* (a locus involving a conspicuous doublet). Lewis finds strong genetic support for the view that in this doublet the gene for Star is in one band of the doublet and that for asteroid (a similar but not allelic mutant) is in the other. He did not succeed, however, in separating experimentally the two bands of the doublet.

Such lines of evidence, taken together, make it probable that a doublet is really a double structure, although there are some grounds for questioning the interpreta-

tion on the basis of cytological complications which can not be gone into here.

Whether the two bands of a doublet are really homologous or not, however, is less clear. Lewis's genetic evidence tends to indicate that they are, but needs to be supported by studies on other cases. We are still far from an understanding of the finer details of organization of the salivary gland chromosomes as seen under the microscope, and such studies, like the genetic investigations just suggested, might contribute greatly to a solution of the present problem. In such studies particular emphasis should be given to any possibility of identifying *qualitative* differences between the two bands of a doublet—either cytologically or genetically. If doublets are single band repeats we need evidence of incipient evolutionary change bringing about a difference between the two loci. Needless to say, such evidence will be very difficult to secure or interpret.

Almost the same problems as those just considered are presented by the pairs of bands which do not show the doublet configuration in the salivary gland chromosomes. Are these pairs simply chance associations of similar bands, or do they represent doublets in which the synaptic attraction has disappeared because of qualitative divergence of the two loci? Rapoport expresses the latter view, based on experimental studies on the Bar duplication, in which he secured multiple duplication of the Bar region up to sextuple and octuple. He goes even further than the pairs of bands and makes the proposal that chromosome regions in which several similar bands lie in sequence represent multiple duplications of one original band. Correlating the additive genetic effects with the chromosome changes in the Bar series, he develops the interesting hypothesis that multiple repeats offer one possible explanation for orthogenesis—particularly cases of orthogenesis which paleontologists have regarded as involving especially rapid evolution.

In this connection, reference should be made to the

subject of heterochromatin—that ill-defined, yet bulky, chromosome material apparently present to some extent in all chromosomes. As you know, it is associated with the so-called inert chromosome regions, which appear to be almost devoid of genes and which are relatively more prominent in the mitotic cells than in the salivary glands. The behavior of this material suggests that it may have been derived through large-scale duplication of one or a few ancestral units, and that it may possibly be simpler or more primitive than the so-called "euchromatin."

Another line of evidence, pertinent at this point, comes from several studies on maize, especially those of McClintock and Stadler. I can only cite one—an investigation by McClintock which involves both cytological and genetic analysis. McClintock was able to identify two very short, adjacent segments near the tip of chromosome 9 in maize. Loss of the distal one resulted in a chlorophyll deficiency giving pale-yellow seedlings. Modification (or, presumably, loss) of the other likewise caused a chlorophyll deficiency—giving yellow-green seedlings. Simultaneous loss of both segments gave complete chlorophyll deficiency—white seedlings.

The significant aspect of this situation is the fact that we have adjacent chromosome regions influencing in an additive manner the production of a quantitative character—chlorophyll intensity. There are other similarities in their action which I have not included here. On the basis of her results, published in a series of papers, McClintock has developed an unpublished interpretation which she kindly permits me to cite here. On this interpretation, the regions under discussion near the tip of chromosome 9 are regarded as made up of a series of similar or identical genes, each of which contributes a specific quantity of substance necessary for chlorophyll production. They therefore have an additive effect. Such a series would be interpreted as having arisen through duplication of individual loci or short chromosome segments. The evolutionary possibilities in this

case are evident—and, of course, one is tempted to apply them to the whole problem of the origin and evolution of chlorophyll production in plants.

If we wanted to turn back to Drosophila we could likewise find evidence from various genetic studies supporting the thesis that adjacent chromosome regions or loci often have similar genes and may represent duplications, as Schultz has pointed out. The Star-asteroid case investigated by Lewis is cited only as a particularly clear-cut example.

Obviously, studies such as those just cited are only suggestive; but they open up a large field for investigation and speculation. Suppose we assume that the series of similar single bands found here and there in the salivary gland chromosomes do represent multiple repeats—then what about other bands in the chromosomes which resemble these particular ones? Are they homologs also? They could readily have been separated from the series through inversions of larger segments of the chromosomes. From this it is only a step to the grouping of all the bands of a chromosome into a few classes on the basis of their morphological similarity and implying that all those in each class are homologous—with the result that we would have only a few kinds of genes and have many representatives of each kind, with minor grades of difference within the classes. Thus we could reach almost any height of speculation.

So much for single band repeats—possible or real. It is something of a relief to turn from them back to the category of duplications we began with—the ones we know are duplications. These exhibit clear-cut band patterns which can be matched, and in favorable cases they exhibit the lateral synaptic attachment, band for band, which is regarded as the final evidence of homology.

Before considering other actual cases of duplications in detail it may be useful to look at some of the general characteristics and potentialities of different kinds of duplications. In the first place, of course, to be of evolu-

tionary importance a duplication must be such as to permit survival of the cell and the organism. Consequently, we would expect repeats that are established in natural populations to be reasonably small, at least in higher organisms, because otherwise they would be apt to upset the genic balance. Evidence from the salivary gland chromosomes tends to fulfill this expectation. Secondly, if the duplication is to persist and function in evolution, it presumably must have or soon acquire a beneficial function—for example, in ordinary plants an increase in chlorophyll production, such as that detected by McClintock in maize. In the third place the duplication must be such as to become stabilized in the population or species. Some types of duplications have the latter characteristic; others do not. This may be illustrated by referring again to the Bar case. The Bar duplication is inherently unstable and shows its instability, as already noted, by giving rise to higher multiples which in turn may revert back to lower ones and even to the original wild-type condition. The reason for this is that the repeated regions are adjacent to one another and are in series—*i.e.*, are oriented alike—not reversed in sequence. Consequently, unequal crossing-over can readily bring about the changes just noted. In natural populations this kind of duplication is conspicuous by its absence. Instead of it we find the so-called "reversed repeats" when the repeated regions are adjacent.

As other authors have pointed out, the stability of reversed repeats is due to the fact that unequal crossing-over here leads to non-viable chromosome conditions—*i.e.*, modifications that do not persist—which is not necessarily true in the serial repeats like Bar.

This situation presents interesting possibilities. The characteristic is both beneficial and detrimental. It tends to insure the required stability of chromosome organization; but at the same time it reduces the number of viable germ cells produced in proportion to the amount of crossing-over. This raises the question as to how many re-

peats and how large ones an organism can tolerate without damage from this non-viability. I am sorry time does not permit exploring this question further.

Another question raised here concerns the matter of single band repeats. If serial repeats are inherently unstable how could a series of two or more single bands, such as doublets, pairs or higher multiples, become stabilized? We might assume that when only two bands are present one is reversed (as suggested by Lewis) and that this gives stability; but so far as I know we have no evidence that such reversal would have this effect here, and in any event it would help us little where four or more bands are involved.

Further interesting information about repeats comes to light when we compare conditions in different organisms, and when we examine certain individual cases. Take, for example, the condition found frequently in wild populations of Sciara, in which one band of what appears to be a doublet in one chromosome of a pair is lacking in the other member. This condition is found frequently in wild populations of Sciara, but seems to be exceedingly rare in Drosophila. Should we infer that evolutionary change in this respect is going on relatively rapidly in Sciara? Unfortunately, no obvious external modifications of the flies accompany these conditions, so we have no evidence as to whether any one condition is more valuable to the organism than the others.

In the case of the larger duplications (as well as the doublets, etc.,) there is evidence in both Drosophila and Sciara that in very closely related species conditions are similar, especially in cases where hybrids have been secured. But exact information on this point is relatively meager thus far, because of technical difficulties which I will come to in a moment. In Drosophila, according to Dobzhansky, there is a tendency for repeats, when found, to be concentrated in particular chromosomes. And even when species are compared that are not closely enough related to be hybridized, these par-

ticular chromosomes are found to be ones which, on other grounds, are considered to be homologous in the different species—which suggests common origin and evolutionary relationship. In Sciara, a similar indication is seen in the repeats found in the X chromosome of several species. This situation is under investigation at the present time, and one aspect of it warrants special notice here, even though the study is far from complete.

In at least four species of Sciara the X chromosome exhibits a new type of repeat condition—one in which the repeated region appears to be represented three times in different parts of the chromosome. Two of these species, *S. ocellaris* and *S. reynoldsi*, are very closely related and will hybridize. In them, the "triple" repeats have been studied intensively. The technical difficulty of getting good figures is greater than ever here where there are two sets of synaptic attachments. The stresses resulting from these attachments when the "smears" are made, almost invariably distort the pattern in the repeat regions. Hence, unfortunately, some points are not entirely clear as yet; but the general aspects seem to be evident. The triple repeat conditions in the two species seem to be identical and the following description applies to both. As the photomicrographs (Figs. 1 and 4) show, the X chromosome is typically in the form of a figure 8 in which both ends of the chromosome are folded back. A short region near end-1 is attached laterally to the two other parts of the chromosome which lie very close to it in the figure, thus holding the chromosome in this configuration. The attachments are shown in Fig. 4. Some of the bands near end-1 are continuous at the left with bands not far from end-2 and the same bands extend out to the right connecting with bands in the third repeat region. Further details which do not show in the photomicrograph appear to be as follows. The repeated segment involves four bands, no two of which are exactly alike. Each of the three segments has the same pattern. Synapsis apparently occurs between all four bands in one segment and the corresponding ones in another.

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For convenience I have designated the segment near end-1 of the chromosome (shown in the middle) as R-1 and the other two as R-2 and R-3 respectively.

At first it was assumed that the synaptic relationship between the three repeat regions here would be alike.

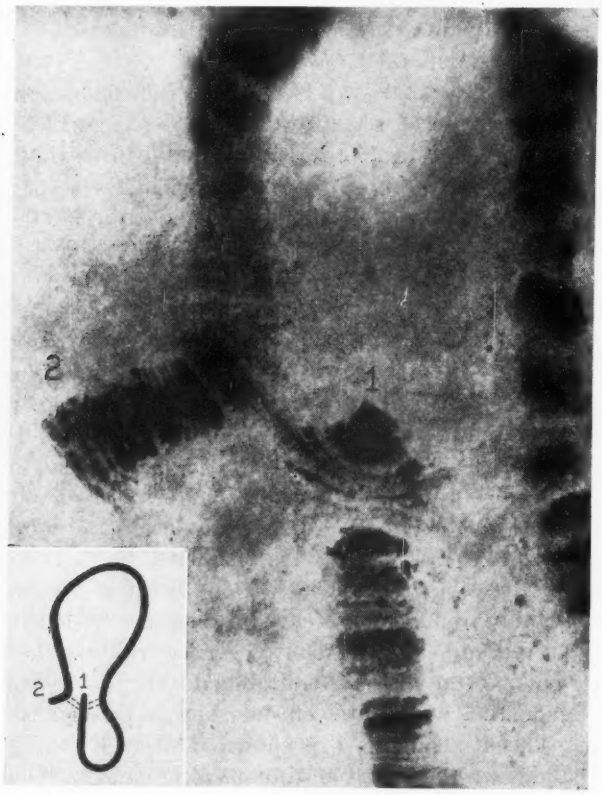

FIG. 4. Photomicrograph and explanatory diagram showing the "triple" repeat condition in the X chromosome of *Sciara ocellaris* Comstock. See also Fig. 1. Explanation in text. ×1500.

But not so. Examination of hundreds of figures has shown that apparently without exception synapsis occurs only between R-1 and R-2, and between R-1 and R-3, but not between R-2 and R-3. This is true in both species.

Such selective synapsis seems extraordinary, for we know from evidence from triploid conditions in Drosophila and elsewhere that in the salivary glands three homologous chromosomes can associate uniformly and intimately. Even if we assumed that for some reason synaptic attraction in the Sciara case were satisfied by a two-by-two association we would expect random union between the three repeat regions. Regions 2 and 3 should unite as frequently as either does with region 1. Unless our observations are at fault, therefore (a possibility which can not be ruled out at the moment), we can only conclude that there are different degrees of homology here in spite of what appear to be morphologically similar patterns. On a qualitative basis R-1 and R-2 have something in common which results in synapsis; similarly, R-1 and R-3 have something in common, but a different something from the other; but R-2 and R-3 do not have either quality in common. Are we to conclude from this that R-1 represents an ancestral condition from which R-2 and R-3 have evolved qualitatively in different directions? At the moment this seems to be one of the possible interpretations. In any event, the present condition presumably came into existence before the two species became distinct from one another. In other words, not only was the triple repeat present in the ancestor of the two species, as we have suggested in earlier papers, but the qualitative or other changes must have taken place in that species or in its ancestors, unless we make the improbable assumption of independent origin.

Not enough is known as yet about the triple repeats in the other two Sciara species just mentioned to warrant more than the statement that the size and the location of the repeat regions are such as to suggest the possibility of a common origin with the regions just considered.

A second feature of the triple repeat case is what may be called the unit action or behavior of the repeat region. In the triple repeat, why should the three regions be the same size? In other words, why should each have re-

mained complete and intact over such a long period of time? It looks as if the region behaves as a unit either in the sense that it can not be fractionated, which seems improbable, or that it performs a unit function for which all parts are essential. The synaptic behavior points in the same direction. Is there a primary locus or gene in the segment which acts as a controlling agent, but which requires the presence of the others for proper functioning? And are we dealing with a situation involving position effect, in which the parts must all be in proper relationship to one another? Much could be explained on that basis, not only in the case of triple repeats, but in ordinary repeats. It looks very much as if this unit action may be characteristic of all established "pattern repeats"—*i.e.*, repeats involving more than one kind of band. If these surmises are correct, we may wonder how many other regions in the chromosomes also represent units of action in this same sense.

Such comparisons could be continued at considerable length; but I fear I have already tried to cover too much ground.

And now to recaptiulate and bring together what has been presented: We may distinguish, I think, three types of conditions which have particular interest for us. One type may represent duplications of single loci or disks; this includes the doublets, the pairs of disks and the series of three or more similar disks. If these all represent recent duplications, then apparently all the well-known salivary gland chromosomes are full of duplications—which, on our present view, would not be surprising. Two major uncertainties face us here, however. First, do these conditions really represent duplications? Second, if they do, how did they get stabilized? Why does not unequal crossing over cause great variability? The other two types of conditions represent duplications of larger chromosome regions—they are the "pattern repeats"—the adjacent repeats and the non-adjacent repeats, respectively. The distinction made between them

is arbitrary, but useful. In adjacent repeats a reversed orientation is ordinarily found—presumably required for stability. It is difficult to see how a series of more than two such regions could become established except under special conditions (such as absence of crossing over in the repeated regions). In the non-adjacent repeats, however, orientation appears to be unimportant, and more than two repeated segments may be present (as in the triple repeats in Sciara). Like the single band repeats, this type should be capable of indefinite multiplication; but unlike the single-band repeats these should be capable of attaining stability without interference by unequal crossing over (except under special conditions).

If this résumé is correct, then, we would expect the three kinds of duplications to have different evolutionary potentialities, as just indicated.

Such is the picture, at least as I see it. If I were not past forty, and if I did not have to worry about the inroads of *Merulius lacrymans*, I would present a peroration at this point. As it is, however, I think I had best be content with expressing an opinion. For the most part, the discussion this evening has served to raise questions without giving the answers. I realize that many of the answers are shrouded in uncertainty. I realize also that we have scarcely more than crossed the threshold of analysis of the main problems. Nevertheless, I think much progress has been made in the past ten or twelve years. And I think we now have available both the material and the methods for making much greater progress in the future. So I think the field presents a stimulating challenge to any one who would help unravel the secrets of evolution (especially if he is under thirty).

THE GENETIC ASPECTS OF NATURAL POPULATIONS[1]

ACTUAL AND POTENTIAL GENE FLOW IN NATURAL POPULATIONS

PROFESSOR CARL EPLING
UNIVERSITY OF CALIFORNIA, LOS ANGELES

AMONGST the flowering plants hybridization is apparently a widespread phenomenon, not only between annectant but between sympatric species. The hybrids produced are not necessarily sterile, but may be sufficiently fertile to produce backcrosses and even hybrid swarms. Because of the weak intrinsic barriers which must accordingly characterize such species, the assumption might logically be made that gene flow between them would obliterate the specific differences in morphology. Yet their often sympatric occurrence, as well as the taxonomic treatment usually accorded them, suggest that the constancy of the species as a whole is not affected and that the potential flow of genes is effectively blocked by extrinsic factors which operate in an environment in which the norm of succession, and hence, the equilibrium of competition, is not materially disturbed. But, with a certain degree of compatibility the potentiality of gene flow may remain and might provide a genotype capable of exploiting an abrupt change in the pattern of competition. If such be the case a premium would be put upon the plasticity provided by the incomplete barriers of flowering plants rather than upon complete isolation. The observations which follow suggest the possibility.

Arctostaphylos is a shrubby genus of the heather family of which two well-defined species are *A. mariposa* and

[1] Presented at a joint symposium of the American Society of Naturalists, the Genetics Society of America and the Society for the Study of Evolution, on December 31, 1946, at Boston. A third paper was presented by Professor Th. Dobzhansky on "Changes Induced by Natural Selection in Wild Populations of *Drosophila*." It was based upon a technical article in press and is not included here.

A. patula. Near the boundary of Yosemite National Park (and elsewhere) the former occurs at lower elevations in drier sites, the latter at somewhat higher elevations on more shaded and cooler sites. Their ranges often anastomose and where they meet hybrids are frequent. I am indebted to Professor Stebbins for calling my attention to them and to Professor Dobzhansky for aid in gathering the data.

To study the effects of hybridization a transect of five stations was followed from pure *mariposa* stands to pure *patula*. Along this transect a total of 1,015 individuals was examined. Forty-six, or about 5 per cent. of the total, were classed as F_1s and at least five as putative backcrosses to *A. mariposa*. As a quantitative expression of specific differences, a representative leaf from each individual was measured as to length and breadth and the proportions ascertained. This is admittedly a crude device, but it can be said that the results agree with observation of other characters. The leaf was chosen as being the best available indicator of intermediacy in the hybrids, not only as to proportions but also texture and color. The means of the sample at each station, excluding the hybrids, are shown in Fig. 1.

The hybrids were not only abundant and vigorous, but were setting fruit in about the same proportion as the parents. That some seeds are viable is evident from the probable backcrosses observed. Gradual replacement of the population, and hence opportunity for the establishment of the hybrids, is indicated by the differences in size of the individuals. In other words, a channel for gene flow would seem to exist. But the data suggest that despite the initial compatibility expressed by the large proportion of hybrids, both species are maintaining themselves. Neither is apparently affecting the other unless it be at a level difficult or impossible to distinguish from parallel variation. At station 4, where intermixture was greatest and where hybridization would be expected to obscure the specific differences, the sharp-

est differences were found on the whole transect, exclusive of the hybrids themselves.

The second example to which I shall refer are two species of *Salvia*, a half shrubby genus of the mint family. *S. mellifera* and *S. apiana* are sympatric over a large area, *S. mellifera* tending to be coastal and more mesic in its requirements. *S. apiana* extends further into the interior and is more xeric. The extensive area which they occupy in common and their present spatial relations and associates indicate that the species have been in con-

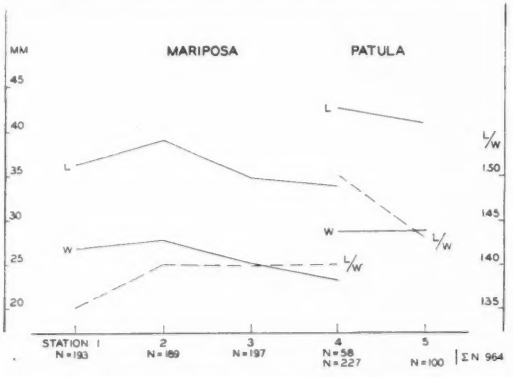

FIG. 1. Illustrating the size and proportions of selected leaves of *A. mariposa* and *A. patula* at 5 stations along a transect (see text). Scale at left: absolute mean proportions as shown by unbroken line. Scale at right: ratio of length to breadth, as shown by broken line. Both species were intermixed at Station 4.

tact for a long period. The somatic number of chromosomes is the same: $2n = 32$. The karyotypes are similar.

Hybridization is frequent and may be expected at any point of contact. The hybrids usually found are F_1s, but small hybrid swarms sometimes occur. In these the F_1s are few and the backcrosses (or segregates from selfing) are relatively abundant. But they form only a small proportion of the whole population. The fertility of the F_1s is low. After open pollination, which is accomplished chiefly by various species of bees, the visibly good seeds are at most 2 per cent. of the total possible. Because of

differences in flower structure with reference to pollinating agents, natural backcrossing is largely restricted to *S. mellifera*. These backcrosses are vigorous, both in the garden and in nature. Their seed set is increased to as much as 30 per cent. The second backcross is frequent, but difficult to distinguish from *S. mellifera*.

In this example, the initial compatibility is low. But the fact that viable combinations are obtained in the F_1 and that fertility is increased by backcrossing, indicates the achievement of a channel for gene exchange on a considerable scale. But the evidence suggests that other factors effectively block any potential flow and localize the effects of hybridization.

Salvia mellifera and *S. apiana* differ sharply in the characters which distinguish them, both qualitative and quantitative. Two definite characters which show the greatest differences and at the same time are measurable are the proportions of the corolla and the number of terminal whorls of the inflorescence. They were chosen for detailed comparison, it being expected that they would disclose any visible effects of hybridization. Analysis of these characteristics were made in 16 colonies which range from Concord, near San Francisco Bay, far beyond the range of *S. apiana*, to the San Jacinto Mountains near the center of its concentration.

Amongst these colonies, I shall consider, first, a mixed colony on Sunset Boulevard in Los Angeles. Two hundred and seventy-six individuals of this colony were tagged for study, of which three were classed as F_1 individuals, fourteen as backcrosses and an uncertain but probably larger number as second backcrosses. All grew in the vicinity of the *apiana* plants, but with *mellifera*. They were apparently of different ages. About two-tenths of a mile on either side of this colony were two other colonies, pure for *mellifera*. Samples were obtained from each by following a rough transect through them and the proportions of the corollas and the number of terminal whorls were recorded. Comparison of the

means of each colony, exclusive of the hybrids, is shown in Fig. 2. Considering first the corolla proportions, it will be seen that the mean of the mixed colony, exclusive of the hybrids, is less than that of one adjacent pure colony, but more than that of the other (Sepulveda). Comparing these means, now, with those of other colonies, both within and outside the range of *S. apiana*, it will be seen that the San Jacinto colony, the Sepulveda and Mulholland colonies and the San Benito colony are essentially the same both in mean and in range. In other words,

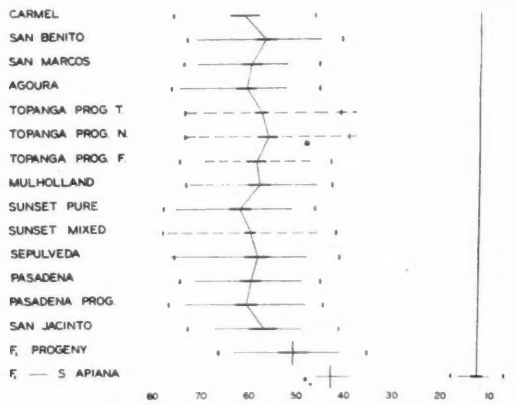

FIG. 2. Ratio of length of upper lip to lower lip of the corollas of *Salvia mellifera*, by colonies, geographically arranged from north to south. The observed range (horizontal line), its theoretical limits (3 σ, vertical bar), the mean (connected by vertical line) and its theoretical limits (horizontal bar, 3 σ) are shown for each colony. Below, the same for *S. apiana* (one colony), 3 F_1 plants (Sunset mixed colony) and garden progeny of one. Broken lines indicate colonies in which hybrids were found.

variation between samples is about what one would expect as an expression of intercolonial variation, modified no doubt by imperfections of sampling. Essentially the same information is given by the whorl number, and also by the coefficient of variability of both characters.

Again, these are admittedly crude measures, but the results do reinforce the same conclusions drawn from comparison of other characters, that *S. mellifera* and *S.*

apiana are two sharply differentiated populations despite the degree of their compatibility in nature. Any influence of one upon the other is at a level where it can not be distinguished from parallel variation between two related species, at least with the methods available.

The same conclusion can be drawn from a contrasting physiological character, the time of first flowering. *S. mellifera* flowers earlier, *S. apiana* later, but their periods overlap. Records of tagged plants show that although individuals of the mixed colony not otherwise distinguishable from *S. mellifera* may come into flower long after their normal period, those of the adjacent pure colonies show no such tendency.

As a test of the localization of hybridization, progenies were grown from each of 10 wild plants taken along a transect of approximately 100 yards in a mixed colony in Topanga Canyon, leading away from a stand of *S. apiana*. The differences in range and mean of the progenies of the 5 plants nearest to (N) and furthest from (F) *S. apiana* are shown in Fig. 2. The data suggest that the radius of effective transport of pollen and seed is relatively short.

The San Marcos colony is also instructive. Most of the plants measured were 2- or 3-year-old individuals which had come into a burn. Adjacent were old *mellifera* and *apiana* plants established before the burn. No evidence exists, to be sure, that hybridization was in progress before the fire, but in view of its prevalence when the species meet, it would be expected. Yet, in the new generation there is no evidence of *apiana* influence, for the San Marcos colony does not differ significantly in corolla proportions from the San Benito colony, 100 miles north, or in whorl number from the Concord colony near San Francisco Bay.

The facts thus far obtained argue, I believe, that although hybridization may be continued when and where the species come into contact, gene flow from one to the other appears to be locally blocked. Still another fact

supports this belief. *S. apiana* is known to hybridize not only with *S. mellifera*, but with 6 other species which are more than usually diverse and well defined. Except for one uncertain case, I have seen no evidence to suggest that any has been affected by hybridization. This phenomenon is not unfamiliar to plant systematists and a number of examples similar to *Salvia* and *Arctostaphylos* have been reported, but not always documented from this standpoint.

I wish now to refer briefly to another and different situation which exists between 3 sympatric diploid species of *Delphinium*. Investigation of this genus by Dr. Harlan Lewis and myself is still in progress and incomplete, but enough data are at hand to justify some tentative conclusions. The chromosome number is the same for each, $n = 8$, the karyotypes are similar and pairing in F_1 individuals is as regular as in the selfed parents. The per cent. of visibly good pollen is generally high. In a given mixed colony, the flowering periods overlap to varying degrees. Mean seed set of F_1s selfed in the garden ranges from about 15 per cent. to 25 per cent. of the selfed parents. Backcrosses are slightly more fertile. F_1s, F_2s, backcrosses and sister crosses, so far as we are acquainted with them, are as vigorous and normal as species selfs. Seed germination is good.

With this degree of compatibility it is difficult to understand how these three species maintain themselves. But more than that, despite careful search in mixed colonies, we have as yet found no evidence even of initial hybridization, although the F_1s would readily be detected. The ecological differences are slight, incomplete experiments indicate no marked pollen preference, incomplete study of the pollinators by our colleague, Professor Gorton Linsley, indicates no marked insect preference. The potential flow of genes seems assured, yet it is apparently not realized, or if so, to an extent far less than might be expected in view of the compatibility of the species. We can as yet offer no satisfactory explanation of the factors involved.

The data I have reviewed justify two conclusions: first, that each pair of species considered is isolated by the combined action of several mechanisms, of which the incompatibility of their genetic systems is only one; second, that in any given situation the relative effectiveness of the various mechanisms may differ. In *Arctostaphylos* the proportion of F_1s is high, relative to the parents present at the zone of intermixture and to the backcrosses. In *Salvia* the F_1s are few, but the backcrosses may be locally abundant and their fertility increased sharply. In *Delphinium*, although the species are compatible to a considerable degree, little or no hybridization occurs in nature.

Bearing these facts in mind, I believe that the obstructions to gene flow must be sought in isolating factors other than those which incapacitate the hybrid, and particularly those which affect the dispersal of the organism, and the factors which allow it to become an integral part of a natural community.

The production of viable seed would depend primarily upon the cross compatibility of the genetic systems. But the number of hybrid seeds would be reduced by seasonal isolation and by structural differences or insect preference which reduce cross pollination. Limitations on pollen dispersal and seed dispersal would tend to localize the hybrids. The number of hybrid seeds subsequently thrown into competition with the parental seeds would be relatively small. The number expected to survive chance loss, by seed-eating animals, for example, would be smaller. Observation suggests that most seeds are destroyed. There is no reason why these factors, so closely linked to the environment, may not become as effective in reducing gene flow, as those which reduce the viability of the hybrid zygotes, once they become a part of the integrated genetic system of a plant species, which is adapted to the pattern of competition in a closed community of which the norm of succession has not been disturbed.

But given sufficient disturbance of the successional equilibrium of a community and hence an alteration in the pattern of competition, I believe that potential gene exchange previously blocked may then become actual and result either in the modification of one or both species hybridizing, or in the production of a new species by a process as rapid as that of amphiploidy. This, I believe, is the more probable explanation of the origin of two diploid *Delphinium* species, one of which I shall describe very briefly.

Whilst studying the morphology of *Delphinium*, Dr. Lewis observed in 1941 that in *D. gypsophilum* are combined the characters of two species between which it lies both geographically and ecologically. These species, *D. hesperium* and *D. recurvatum*, were accordingly crossed. The initial seed set is approximately normal and germination is good. The F_1 populations are intermediate and somewhat variable. Amongst them have appeared individuals which, should they be met in the field, would be assigned without doubt to *D. gypsophilum*. The strong resemblance between the hybrids and *D. gypsophilum* and their somewhat increased compatibility with it, as well as its intermediate geographical and ecological relationships suggest its hybrid origin. The seed set of the F_2 generation increases to about 25 per cent. of the selfed parents. Last spring, however, one individual when selfed and backcrossed set nearly the mean of the selfed parents. Its fertility was greatly increased, relative to the F_1. When the progeny of this plant are known, more certain conclusions can be drawn. But the facts meanwhile seem to confirm the hypothesis made by Professor Stebbins that isolating mechanisms may be genetically independent of the morphological changes which produce visibly good species. "If genetic isolation is accomplished by the accumulation of small units, genetically independent of morphological differences, these units should occasionally recombine in new ways in the progeny of a partially sterile interspecific hybrid. Some of these

new combinations could be fertile types which would be different in appearance from either of the original parents and exhibit partial sterility with both of them. They would thus become the progenitors of new species."

This is a prophetic explanation of what has seemingly happened in the cross *D. hesperium* × *recurvatum*. Since the fertile recombinations formed in this way can be intermediate in morphology, it is reasonable to suppose that some might also be intermediate in ecological response. Should, then, a new habitat be at hand into which they might enter, thus reinforcing the partial incompatibility with the parent species, the geographical and ecological status of *D. gypsophilum* could be achieved. The recession of the Pleistocene sea which occupied parts of the Central Valley, and the attendant climatic changes might have provided the habitat. Experiments have satisfied Dr. Lewis and myself that hybrid *Delphinium* seeds of diverse origin are able to establish themselves and come to normal flowering and fruiting when broadcast in a new disturbed habitat. I believe, therefore, that there is a strong probability that *D. gypsophilum* arose by hybridization of two diploid parents in the way so brilliantly conceived by Professor Stebbins.

Summary

The morphological constancy of sympatric species may be preserved despite compatibility and hybridization sufficient to allow gene flow, so long as the equilibrium of competition is maintained. Such hybridization might be expected to result in selection for greater isolation. On the other hand, the potentiality of gene flow thus preserved might prove advantageous and provide a genotype which could exploit an abrupt change in environment.

HEREDITY OF GEOGRAPHICALLY AND ECOLOGICALLY ISOLATED RACES[1]

DR. JENS CLAUSEN, DR. DAVID D. KECK AND
DR. WILLIAM M. HIESEY
CARNEGIE INSTITUTION OF WASHINGTON, STANFORD UNIVERSITY

MOST plant species are composed of races, which are aggregations of scattered local populations that have certain fundamental characteristics in common. Some of these races can be recognized on sight, because they are morphologically different; others look alike but are physiologically so distinct that they can occupy different climatic zones; still others are both morphologically and ecologically distinct. Some races are very local; others, of wider distribution. Some seem to have arisen through chance spatial isolation from other populations of the species, whereas those in ecologically different environments appear to have evolved in response to the selective effect of these environments. Geographically and ecologically isolated races are biological units just below the species level. They are of evolutionary importance, because they are probably often the forerunners of new species. Very little experimental evidence on the heredity of such races is on record. In the following account, the genetic basis will be discussed of four sets of races that exemplify kinds of intraspecific differentiation.

GEOGRAPHIC RACES IN HEMIZONIA ANGUSTIFOLIA

The coast tarweed of California, *Hemizonia angustifolia* DC., is a 10-chromosome species of the Madiinae of the sunflower family. It occupies a narrow strip on the coastal side of the outer Coast Range extending inland only as far as the coastal fog. It is composed of two major races: one occupies a coastal strip 275 miles long from northern California to south of Monterey Bay; the other starts 40 miles farther south and extends over a

[1] Presented by Dr. Clausen at a joint symposium of the American Society of Naturalists, the Genetics Society of America and the Society for the Study of Evolution, December 31, 1946, at Boston.

distance of 40 miles. It is separated from the northern race by the Santa Lucia Mountains, which rise precipitously from the ocean, leaving no coastal plain for this species to occupy. Geographically the two races are therefore effectively separated, but ecologically their habitats are similar.

Small but significant morphological differences separate the two races of the coast tarweed. These were studied in 11 populations from different localities of the northern race and 5 of the southern grown in a uniform garden. Some of the differences between the two races can be seen from the upper two plants in Fig. 1. The plant of the northern race, left, is of a population from a grassy hillside west of Belmont, San Mateo County, south of San Francisco on the peninsula; this race has a low and broad habit, slender and open branching, and relatively small heads. The plant of the southern race, upper right, from a population originating in fields close to the sea 15 miles north of San Simeon, San Luis Obispo County, has more erect and robust branching, and larger, more congested heads. These differences are small, as compared with the morphological characters distinguishing most species, but they are fairly consistent, and by some authors the two races, subsp. *typica* in the north, and subsp. *macrocephala* in the south, have been considered as distinct species.

The two forms are easily crossed and their hybrid is as fertile as the parents. A second generation of 1,152 plants was grown, and the segregation indicated that each of the small differences between the parents depended upon the action of multiple genes, each with a small additive effect. In this manner, the slight morphological differences between the races were resolved into even smaller steps. The recombinations of these genes produced great variation in the F_2; no two individuals were alike, and no plant exactly like either parent was recovered from this sample. The six F_2 individuals shown in Fig. 1 represent various types found. The center individual in the upper row combines the low habit of the

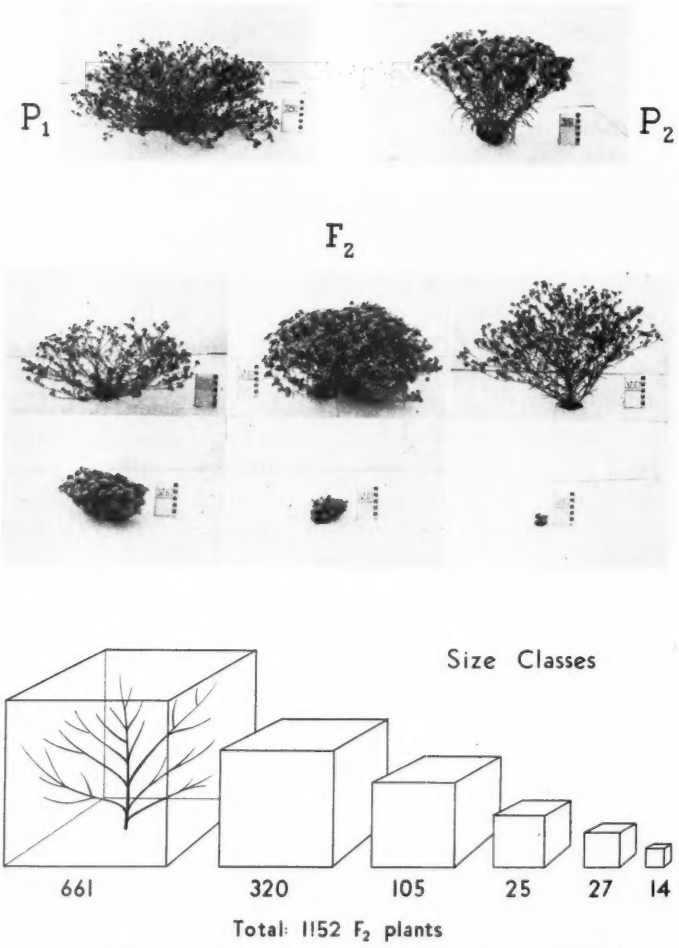

FIG. 1. Segregation in a hybrid between geographic races of *Hemizonia angustifolia*. P_1, subsp. *typica*; P_2, subsp. *macrocephala*; F_2, three vigorous and three substandard segregants. The scale is 10 cm. high. The size classes are represented by cubes, the edges of which are 50, 35, 25, 15, 10 and 5 cm., respectively.

northern race with the density of the southern, but its heads are intermediate in size. The individual in the same row to the right has the erect habit of the southern and the open branching of the northern race.

The second generation offspring were generally vigorous, although many were somewhat weaker than the parental types. All F_2 plants were classified according to their greatest dimension as represented by the size of the cubes in the lower row of Fig. 1, shown to the same scale as the plants above. More than 57 per cent. of the offspring (661 plants) were in the largest size class, ranging from 35 to 50 cm in greatest dimension, as large as the parental races. The plants of this class were similar in size to those illustrated in the upper row of the F_2.

The substandard groups, represented by the five cubes to the right, contained nearly 43 per cent. of the plants. They ranged from 35 cm down to less than 5 cm, and are represented in the lower row of the F_2. During the 7 to 8 months that these plants lived, the small individuals remained healthy and continued to flower, although there was little or no increment in their growth. Meanwhile, sister plants increased to 1,000 times the volume of the extreme dwarfs. The genes determining the rates of the physiological processes in the two races are probably not identical, for certain recombinations have resulted in upsetting balanced development. As the two races evolved, their genes became so different that they were no longer completely interchangeable without detrimental effects in the progeny.

The barriers to interbreeding between the northern and southern races of the coast tarweed are nevertheless of a much smaller order than those typical of distinct but related species. So many of the hybrid offspring are vigorous that these racial complexes are not considered to have yet reached the status of distinct species. The northern and southern races of *Hemizonia angustifolia* are therefore classified as geographic subspecies. They are not distinct ecotypes, because both occur in the same coastal zone and react very similarly. The species there-

fore appears to have only one ecotype but two geographic subspecies.

Evolutionarily, the two races are in the transitional stage between subspecies and full-fledged species. Many small steps were required to produce these differences, and many genes were involved, but the changes did not affect the interfertility of the races or the structure of their chromosomes. Races in such transitional stages of development are significant, because their existence indicates gradual steps in the evolution of species.

A New Genus—or Just a Subspecies?

An example of a geographically isolated race of very limited distribution is afforded by a rare plant of the sunflower family that was discovered in 1940 in the inner Coast Range of California near the quicksilver mining town of New Idria. It is a little spring-flowering annual, 5 to 15 cm tall, occurring as a small colony of some 300 individuals on a serpentine hillside. It nevertheless maintains itself there, for it was collected once before at New Idria some 40 years ago, but not classified. This plant is so unlike anything previously named that it was thought to belong to an undescribed genus. Technically it would be assigned to the Helenieae, the sneezeweed tribe, but it fits none of the known genera. There was, however, something about its vegetative characters that suggested the genera *Layia* and *Madia* of the neighboring Madiinae of the Heliantheae, the sunflower tribe, although it has no ray-florets or outer involucre, deficiencies which technically exclude it from that tribe.

In our garden this supposedly new genus with uncertain tribal affiliation was provisionally called *Roxira serpentina,* the generic name commemorating its two discoverers, Mrs. Roxana Ferris and Dr. Ira L. Wiggins, of Stanford University. It has 8 pairs of chromosomes, resembling those of *Layia,* and crossings were therefore attempted between it and 8-paired species of *Layia* and *Madia.* The *Madia* crossings were unsuccessful, but vigorous hybrids were obtained in crossings with two

Layia species. The hybrid with *Layia glandulosa* was even completely fertile, and the 8 chromosomes from the two supposedly distinct genera paired perfectly, indicating a very close evolutionary relationship.

Layia glandulosa (Hook.) H. & A., the desert *Layia*, is a species of wide distribution in sandy habitats. It occurs from Lower California to central California on the coast side of the mountains, and from Arizona to Washington in the desert regions of the Great Basin. Its common form, Fig. 2, upper left, has 8 showy, white rays and long, shining white pappus-bristles on the disk-akenes. *Roxira*, upper right, is rayless (*i.e.*, discoid), and it is a smaller and much more slender plant, with stubby and dull brown pappus scales. The F_1 hybrid, second row, is a tarweed in good standing, for it meets the technical requirements of the Madiinae, having 3 to 8 rays per head with accompanying ray-bracts that in turn enclose the ray-akenes. However, some of the heads on the same plant may have no ray-florets at all.

More than 1,200 F_2 plants were grown. They were generally more vigorous than nonhybrid forms of the desert *Layia* or than other hybrids between races of that species. The segregation was very striking, as seen in the lower half of Fig. 2. Forms with 8, 5, 3, 1 and no rays were segregated, and the rays were long, short or very short. The akene-enfolding bracts always accompanied the rays. The color and length of the disk-pappus varied from one parental extreme to the other.

The segregation for just three of the 9 to 10 characters that distinguish the parental forms are listed in Table 1. Each character was difficult to classify, because the parental differences were resolved into so many small steps that a completely graded series of variants was obtained. It was estimated that between 10 and 20 pairs of genes were possibly involved in the segregations, but both of the parental morphological types were nevertheless recovered in the F_2. Considerable genetic linkage was evident as demonstrated by the data in Table 1. For example, the short pappus typical of *Roxira* was found

mainly with no, few or short rays. It appears that one or two pairs of genes may determine the presence or absence of rays and bracts, but several pairs determine the number and the length of the rays, and about three pairs the differences in length of the pappus. *Roxira* is evidently

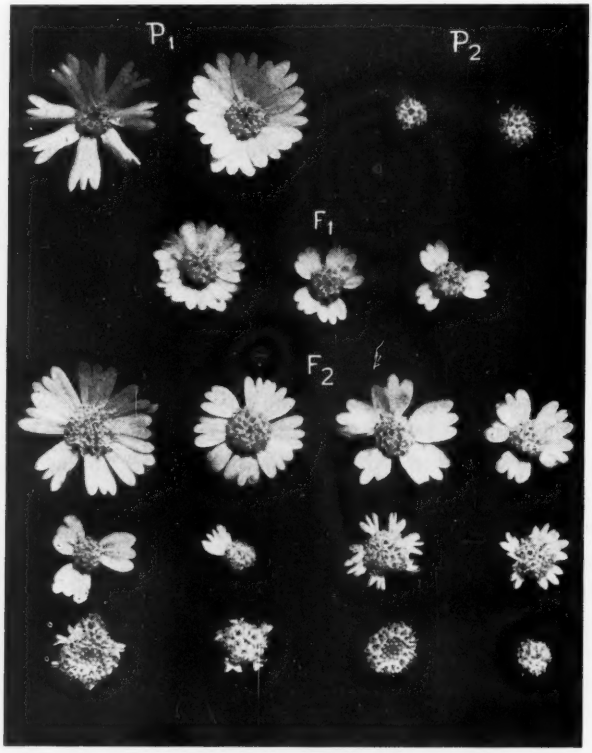

FIG. 2. Segregation in a hybrid between *Layia glandulosa* and *Roxira serpentina*. Flower-heads of two individuals of *Layia glandulosa* (P_1), upper left, and of *Roxira* (P_2), upper right. Second row, variation in F_1. Three lower rows, segregation in F_2.

predominantly recessive as compared with *Layia glandulosa*.

Although *Roxira* has no ray-florets, it carries genes that shorten the rays of *Layia glandulosa*. Likewise, it

has genes that affect the color of the rays, for 107 out of 988 F_2 plants had pale yellow rays instead of white.

Because of this genetic evidence, *Roxira serpentina* is now considered to be only a subspecies of the desert *Layia* and is to be reduced to the much less glamorous name of *Layia glandulosa discoidea*. A series of systemic changes that would otherwise denote tribal differences, and which on their face value would be worthy of generic rank, have taken place in this species without affecting the interchangeability of the genes or the chromosomes. The two

TABLE 1

SEGREGATIONS IN F_2 OF *Layia glandulosa* × *Roxira serpentina*

Rays		Pappus length			Total		Approximate ratio
Number	Length	Long	Medium	Short			
8	long	42	28	..	70		
	medium	23	51	..	74	157	
	short	3	10	..	13		13
1–7	long	34	37	..	71		
	medium	88	363	5	456	831	
	short	51	247	6	304		
none		16	219	15		250	3
Total		257	955	26		1238	
Approximate ratio		63		1			

forms must have been separated for a long time for such differences to have evolved. No form of the desert *Layia* grows with the serpentine plant, the nearest known colony being 6 miles away on the other side of the mountain range. There is no way of determining which race was first, the rare, inconspicuous one from the serpentine, or the common, showy one from sandy habitats.

Layia glandulosa discoidea has the characteristic of a relict form that in the past may have been much more widely distributed than now. Its presence on serpentine soil instead of on sand may indicate that *discoidea* is an edaphic ecotype and not merely a very local geographic race.

MARITIME AND INLAND RACES OF LAYIA PLATYGLOSSA

In contrast with the preceding two examples of geo-

graphically isolated races is another of ecologically isolated races in the tidy-tip, *Layia platyglossa* (F. and M.) Gray. The tidy-tip is a spring-flowering annual that adorns California hillsides during March and April. This species, with 7 pairs of chromosomes, is common in separated colonies throughout the Coast Ranges from the San Francisco Bay region south to Lower California. Farther north it occurs mainly along a narrow coastal strip.

Layia platyglossa contains an inland and a maritime race, shown in Fig. 3. The inland race, which is widespread and much the more common, is early flowering and has a distinct central leader and ascending-erect stems. On the exposed coastal bluffs it is replaced by a race of matted habit, without a central leader, but with horizontal branches from the base of the plant. This race has thick, succulent leaves, and in the experiment garden it flowers two or three weeks later than the inland race, and remains green much longer. The maritime race occurs over a distance of approximately 225 miles along the immediate coast of central California. Although intermediates occur, the two races do not lose their identity.

FIG. 3. Left, maritime, and right, inland races of *Layia platyglossa*, with their F_1 hybrid between them.

The inland and coastal races of the tidy-tip cross easily, and the hybrid is completely fertile. Such hybrids are more vigorous than nonhybrids, and the increased vigor was evident through the third generation. The F_1 hybrid is shown in the center of Fig. 3 and is intermediate in most characters.

A striking segregation takes place in the second and later generations affecting all characters that distinguish

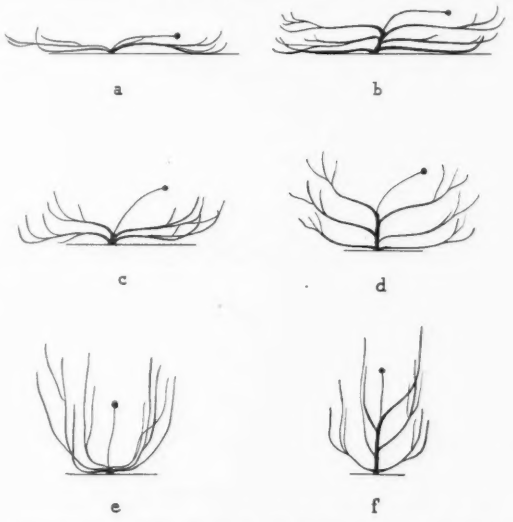

FIG. 4. Segregation in habit in F_2 of maritime × inland races of *Layia platyglossa*. The letters refer to the classes in table 2.

the parental races. The major types of growth habit found in the segregating offspring are indicated in Fig. 4 and the class frequencies of the individuals belonging to these types are listed in Table 2 together with a tabulation of earliness. Separate genes govern the presence or absence of the central leader and the horizontal or erect orientation of the branches. A counterpart of one of the recombinations predominated in one intermediate locality in the wild two miles from the coast but overlooking the ocean. It is illustrated by the plant *c* in Fig. 4 and has

no central leader but merely a central peduncle and long, ascending branches.

About 3 pairs of multiple genes are responsible for the lateness of the maritime form, some 4 to 5 pairs determine the architecture of the plants, and others govern the thickness of the leaves, the number of rays and other characters. The parental types were recovered in an F_2 population of 803 individuals, but they had not become genetically constant. There was a fairly high degree of

TABLE 2

SEGREGATIONS IN F_2 OF MARITIME × INLAND RACES OF *Layia platyglossa*. LETTERS IN PARENTHESES REFER TO FIGURE 4

Branching	Central leader	Earliness		Total		Approximate ratio
		Very late	± early			
horizontal	none (a)	8	38	46	69	1
	present (b)	2	21	23		
± ascending	none (c)	6	221	227	394	15
	present (d)	..	167	167		
erect	none (e)	..	203	203	340	
	present (f)	..	137	137		
Total		16	787	803		
Approximate ratio		1	63			

genetic linkage between the physiological characteristic of late flowering and the horizontal habit of the parental maritime race, as can be seen from Table 2. This linkage tends to keep the ecologically important characteristics together, even though the two races occasionally intercross.

The parental forms of this cross were a maritime race from Point Joe on the Monterey Peninsula of central California and an inland race from a mesa near Etiwanda, San Bernardino County, in southern California. Four additional hybrids between other maritime and inland populations of *Layia platyglossa* have been grown, yielding some 3,600 F_2 individuals. Their pattern of segregation and linkage was similar to that of the hybrid here discussed.

Six F_3 populations of the Point Joe × Etiwanda cross were studied, totalling some 2,700 individuals. Because Layias are self-sterile, these were obtained from mutual pollinations between pairs of F_2 individuals, each pair composed of plants of similar growth habit. All F_3's continued to segregate a great deal, except that two cultures from F_2 plants with central leaders produced only offspring with central leaders, and one from early flowering F_2 plants was early flowering only. On the other hand, three pairs of F_2 plants without central leaders segregated forms both with and without leaders, and five pairs produced offspring that segregated very early flowering to late flowering plants. These results suggest that central leader and early flowering are probably the most easily recognizable recessive characteristics. The gene basis for the two races may be more complicated than the F_2 segregations would lead one to believe. The vigor of the F_3 populations noticeably exceeded that of the parental races, a situation very different from that prevailing in the F_2's and F_3's of hybrids between closely related species, because these are characterized by partial sterility and considerable reduction in vigor.

Hybrids between two maritime races produced only plants of maritime type. More than 1,400 F_2 plants were grown from a hybrid between a maritime *Layia platyglossa* from exposed bluffs near Bodega north of San Francisco Bay, and the previously mentioned form from Point Joe on the Monterey Peninsula. All the F_2 plants of this cross, without exception, were without central leader, were completely prostrate, succulent and late flowering. This result indicates that the genes determining the ecologically important characteristics of the maritime race from the narrow coastal strip are identically arranged in the chromosomes of the parental forms, even though these are separated by a distance of some 140 miles, including the Golden Gate channel.

Another *Layia* species has developed races strikingly parallel to the ones just described. Within a few hundred feet from the maritime *Layia platyglossa* on the

coastal bluffs near Bodega grows a late-flowering, prostrate race of *Layia chrysanthemoides* (DC.) Gray of very similar appearance. The erect, early flowering inland races of both species also mimic each other, although their floral characters differ markedly. It is almost impossible to cross *platyglossa* and *chrysanthemoides* with each other, and their rare hybrids are completely sterile. Their differences are therefore those of very distinct species and are evolutionarily of a much higher order than the superficially more spectacular differences between the completely interfertile coastal and inland races of each species.

Many other species of entirely unrelated families have produced similar pairs of inland and maritime races both in California and other parts of the world. The genetic basis of these, however, has rarely been explored. An example parallel to those in *Layia* is found in the maritime and inland races of *Viola tricolor* L. from Denmark, the inheritance of which has been analyzed (Clausen, 1926, pp. 16–22). Such races are ecologically, rather than spatially isolated. This particular kind is a climatic race, a special kind of an ecotype.

Altitudinal Races in Potentilla glandulosa

Climatic races of a different kind from those determined by distance from the sea are found in perennial plant species that occupy a great range of climates. This is illustrated by the altitudinal races of *Potentilla glandulosa* Lindl., a member of the rose family that is common in the western United States. In central California this species occurs from near the coast to 11,000 feet altitude near the crest of the Sierra Nevada, although it is not found in the great Central Valley. Approximately seven climatic races belonging to four morphologically distinct subspecies of this *Potentilla* occupy the various climatic zones of the transect across central California. A great many forms of these have been tested through the years at the three transplant stations maintained in California

by the Carnegie Institution of Washington (Clausen, Keck and Hiesey, 1940). These stations are located at Stanford University near the coast, at Mather halfway up the Sierra Nevada, and at Timberline near the crest of this mountain range.

Lowland races of *Potentilla glandulosa* differ strikingly from alpine races both in morphological and physiological characters. For example, the robust, large-leaved, small-flowered race from the mild climate of the Coast Ranges, belonging to subspecies *typica*, is in active growth throughout the year; it is resistant to frost, and thus able to grow during the winter when the rains occur. By contrast, the dwarf, slender-stemmed, large-flowered alpines belong to subsp. *nevadensis*, remain dormant over a nine-months' winter in their natural habitat, and then spring into rapid flowering during the brief summers. When transplanted to the lowland station, the alpines are winter-dormant, but for only two or three months, and they become weakened and less floriferous. The Coast Range plants, on the other hand, are unable to survive the long, severe winter at the alpine station.

All the races of *Potentilla glandulosa* have 7 pairs of chromosomes and cross easily. They differ in a great many characters, both morphological and physiological, and although the subspecies by some authors have been recognized as distinct species, their hybrids are completely fertile and vigorous. A plant of the winter-active, self-fertile Coast Range race coming from near sea-level at Santa Barbara was crossed with a plant of the winter-dormant, self-fertile alpine race from 11,000 feet near the boundary of Sequoia National Park, in the Sierra Nevada. The F_1 was more vigorous than either parent, was fairly winter-active and highly self-fertile. A large, vigorous F_2 population was grown at Stanford and segregated for all the 11 or 12 characters that distinguish the parental races.

A striking segregation for the physiological character of winter activity was observed in this F_2. At Stanford,

in mid-January, the coastal parental race has vigorously growing young stems and long leaves, and the alpine is dormant. Among 992 F_2 plants, 183 were classified as very active, having new leaves 10 to 17 cm long, 600 as moderately active and 209 as dormant. A completely intergrading series was observed, giving the impression that the segregation was governed by genes in a multiple series.

Many new forms arose through the recombination of the genes of the contrasting parental races. One of these forms, for example, combined the short internodes of the alpine with the large stem leaves of the Coast Range form, resulting in a short and compacted type with large leafy bracts in the inflorescences. About 3 per cent. of the F_2 belonged to this morphological type. Plants of this form are not found in the wild, but they are of a habit that would appear to be suitable for the extreme maritime environment, which this species has not yet been able to occupy.

An equally striking interracial hybrid of *Potentilla glandulosa* was produced between the foothill and alpine races. One parent came from the foothills of the Sierra Nevada in the scrub oak chaparral near Oak Grove, Tulare County, at an altitude of 2,500 feet. The alpine parent was from a sunny, rocky slope at 10,300 feet, above the Timberline transplant station, north of Tioga Pass in Mono County, on the east flank of the Sierra Nevada.

The foothill race, belonging to subsp. *reflexa*, occupies dry, warm slopes at lower elevations in the Sierra Nevada. It is a tall, self-fertile, large-leaved form with long, divaricately branching stems, open habit, and small, yellow, spreading to reflexed petals. Its plants are susceptible to frost and become winter-dormant even at the lowland station. They reach their best development at mid-altitude, but are unable to survive at the alpine station. The foothill parent used in this cross is shown in the center line of Fig. 5 as it grows in the gardens at Stanford and Mather. This individual was set at the alpine station

seven times, but only once did it survive the first winter there.

The alpine race, in contrast, occupies habitats from around 8,500 to 11,000 feet altitude above tree-line. It is much more frost resistant than the foothill race. The

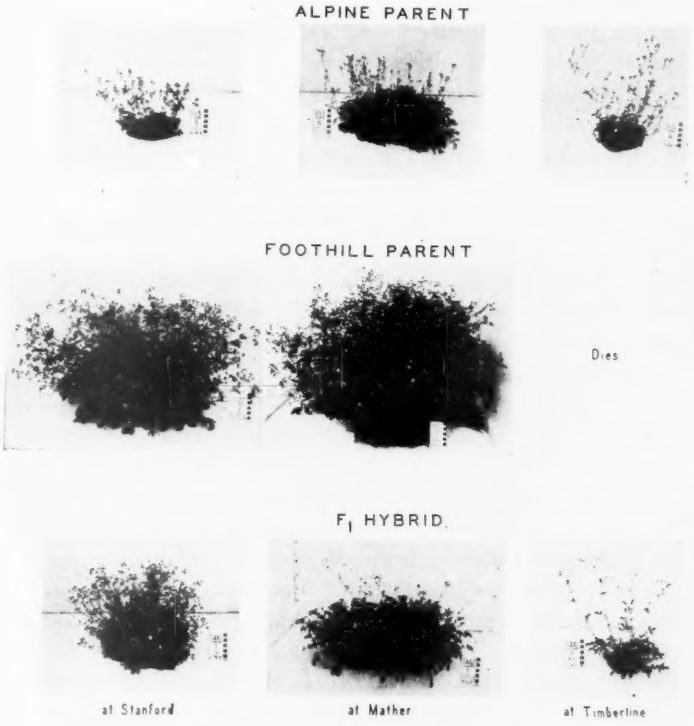

FIG. 5. *Potentilla glandulosa.* Responses of an individual of the alpine and one of the foothill race and their F_1 hybrid in the transplant gardens at Stanford, 100 feet, at Mather, 4600 feet, and at Timberline, 10,000 feet altitude. The height of the scale is 10 cm.

alpine parent is shown in the upper horizontal row of Fig. 5 as it grows at all three stations.

A clone of the F_1 hybrid is illustrated in the bottom row of Fig. 5. The hybrid is intermediate in appearance, but grows vigorously and survives indefinitely at all three stations. It is moderately self-fertile.

All the 575 F_2 individuals of this hybrid were cloned and grown simultaneously at the Stanford, Mather and Timberline transplant stations for at least five years. Detailed measurements and seasonal notes were taken annually on each individual in each environment to determine its climatic fitness.

The parents differ in at least 16 easily observed morphological and physiological characteristics, and in the F_2 a complete segregation in all these characters took place. At each station there is a great variation in vigor, but the plants that are vigorous in one environment may be weak in another, and *vice versa*. Physiological characteristics, such as earliness of flowering, frost resistance and ability to survive in specific environments, are genetically linked with morphological ones such as shape, size and color of petals. Such linkages tend to keep the climatic races distinct even where they meet and intercross. The linkage is not absolute, and many recombinations are found. The nature of the segregation is such as to indicate that each characteristic is determined by several genes. The expressions of the genes in the same individual are modified from one environment to the other. No winter-active forms were segregated in this cross between two forms differing in degree of winter dormancy.

Fig. 6 shows the reactions of three contrasting F_2 individuals in three environments, each horizontal row representing a clone. The plants at the left are those grown in the garden at Stanford, those in the middle at Mather and the ones to the right at Timberline. No two individuals in the F_2 responded to the three environments in the same way, and in this respect the F_2 was much more variable than any wild race. The very contrasting environments of the stations act as a coarse sieve, making it possible to distinguish individuals with different climatic fitnesses. The three plants illustrated represent three very different trends.

The top row shows an individual of typically alpine reactions. It survives at all three stations but is dis-

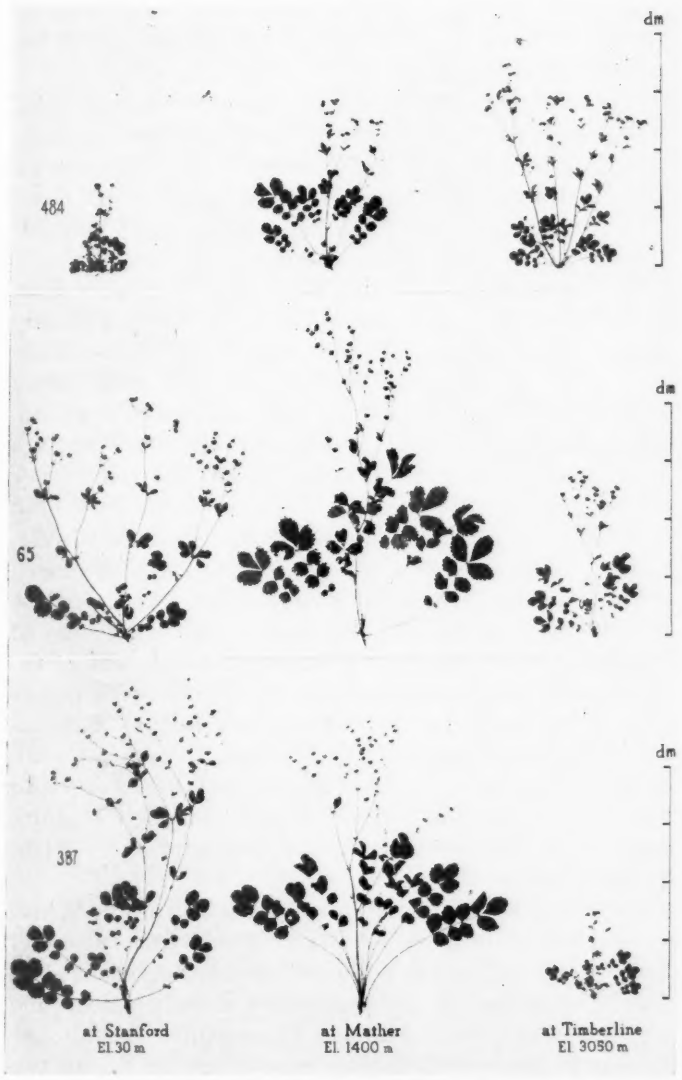

FIG. 6. Responses of three F_2 clones of the hybrid between the alpine and foothill races of *Potentilla glandulosa* at Stanford, Mather, and Timberline. Top row, best at Timberline; center row, succeeding at all three stations; and bottom row, best at Stanford.

tinctly most vigorous at Timberline. Its morphological characters, however, were recombinations of those of the foothill and alpine races.

The middle row shows a clone representing a group of individuals that survive and thrive at all three stations, showing an adaptability not matched by any of the Potentillas from the wild. This individual has the floral characters of the alpine parent and the vegetative of the foothill, except that its herbage is resistant to frost.

The lowest row is of a clone belonging to a group that, unlike either of the parental races, grows most vigorously at the lowland station. In this respect it is similar to the race native to the Coast Ranges, but differs by being winter-dormant at Stanford. Morphologically it resembles the foothill parent. The plant illustrated survives at Timberline, although growing weakly, but many other F_2's of this class die there.

A complete reshuffling of the morphological and physiological characteristics of the two parent races has taken place, resulting in the development of new forms, some of which have potentialities for superior development and others a greater capacity to survive in a diversity of environments than either parent. Half of the F_2 plants have survived for 8 years in the severe climate of the alpine station and many ripen seed there year after year. Some of these even surpass the native alpines in vigor and frost resistance. Many of those that fit the alpine climate so well have vegetative characteristics of the foothill parent.

Eighteen F_3 populations from this foothill-alpine hybrid were grown in the garden at Stanford. They consisted of 3,600 individuals obtained by self-pollinating selected F_2 plants representing very different morphological and physiological types. All these F_3 progenies continued to segregate, most of them as completely as the F_2, but two cultures approached constancy in petal size and color, and some of the others in growth habit. Hybrid vigor was maintained through the third generation.

It is evident from the F_2 and F_3 data that in this cross between a foothill and an alpine race of *Potentilla glandulosa* new climatic races are in the process of development through the recombination of the genes of the old ones. This evidence points to the importance of climatic races from contrasting environments as potential sources of gene material for the evolution of new races.

Conclusions

Both geographically and ecologically isolated races of a species are regulated through the interaction of many genes in a balanced system in harmony with their environment. The genetic and ecologic analysis of hybrids between races from contrasting environments indicates that there are deep-seated functional differences between the races, even though they may appear to intergrade by gradual steps. The differences between natural races are predominantly determined by series of genes of the multiple type, each gene having a minor but additive effect. When crossings occur between contrasting geographic or ecologic races and their genes recombine, so many new forms appear that the variability may far surpass that in the wild populations of the species.

This situation indicates that in nature there is a backlog of unutilized evolutionary resources from which races capable of fitting into many new environments could be synthesized. Hybrids from such crossings are also often superior to their parents in their ability to succeed in a wide range of environments. These facts are of importance in further clarifying the evolutionary pattern in plant life and in furnishing information of importance for those engaged in plant breeding.

Literature Cited

Clausen, J.
1926. *Hereditas*, 8: 1–156.
Clausen, J., David D. Keck and William M. Hiesey
1940. Carnegie Inst. Washington Publ. No. 520.

THE MATING–ANATOMY TECHNIQUE AS APPLIED TO POLYGYRID LANDSNAILS[1]

GLENN R. WEBB
OHIO, ILLINOIS

IT is the objective of the present paper to stress the value of mating-anatomy techniques and studies. This is done expressly to both induce more investigators to explore this neglected field and to present data which may facilitate such studies. Because my experience with the technique has been developed with the study of polygyrid snails, its merits and methodologies are expressed in polygyrid terms. The procedures mentioned are as yet mere crude expediencies; but, even so, are considered to be of sufficient value to justify their present utilization and further extension and development.

In recent years, genitalial studies have become a major point in the evaluation of the taxonomy and biology of the polygyrid snails; and extensive detailed data are available on the anatomy of the retracted organs. Indeed, the entire genitalia is contributive of taxonomically diagnostic features. The sex-organs,[2] however, are especially useful, but the organ of greatest taxonomic utility is the penis. This exhibits wide variation among the groups and species, not only in proportions and basic morphology, but also in the internal structure—exhibiting, papillae, pilasters, variform folds, stimulators, sometimes a verge, and less understood configurations of permanent or transient character.[3]

While the penis is in the retracted-compacted state, these internal structures are very difficult to evaluate anatomically. Frequently adventitious compaction-foldings may be as prominent as true structural entities. The situation is comparable to that of finding an elaborately

[1] Including data on sex-biology, evolution and culturing.

[2] Consisting usually of penis, spermatheca-and-duct, free-oviduct, vagina and atrium.

[3] Archer, Binney, Pilsbry, Simpson and others (see the literature cited).

emboss-ornamented and papilla-decorated sock in a shrunken inside-out condition. In such case, the papillae and other structural parts would have to be differentiated from chance foldings caused by the shrinkage. The retracted penis of polygyrids presents a comparable condition. For these reasons, the only time the true external surface of the penis would be clearly displayed will be during coitus or semen-transfer. Consequently, while the study of the retracted penis (especially longitudinal and transverse sections) reveals obscurely the complex internal structure, the knowledge that this is actually the external surface makes it evident that studies of the anatomy of the sex-organs during functioning may have special value.

Up to now such studies have just begun—the following citations only being known to me on the Polygyridae. Simpson (1901) has described briefly the mating of *Triodopsis* (*Neohelix*) *albolabris* (Say), and has figured an anatomy with partially everted penis. Pilsbry (1905, 1909, 1940) has published figures and brief descriptions of the everting sex-organs of several species of *Ashmunella*[4] in papers on taxonomy and distribution. Archer (1933) has contributed data on the mating-procedure and appearance of the functioning sex-organs of *Allogona profunda* (Say), and of *Mesodon inflectus* (Say); but misidentified parts of the united penises of *inflectus* as an everting vagina. Mention of *Allogona profunda* has also been given by Webb (1943) in regard to stimulator-function, and papers on polygyrid mating-anatomy are pending publication.

Mating–Anatomy Studies

My interest in the mating-anatomy technique and studies began with the incidental witnessing of the courtship and semen-transfer process of *Mesodon elevatus* (Say); wherein the sex-act is completed from a previous

[4] *Ashmunella esuritor* Pils.; *A. levettei ursina* Pils. & Ferr.; and *A. varicifera* (Ane).

condition of insignificant bulbous swelling of the atria; with the extremely swift turgescence; eversion;[5] entwistment; semen-deposition; and separation of the penises. The great increase in turgidity of the extruding parts; the speed of the eversion; the entwistment; and the but-few-minutes-later separation, deturgescence and retraction; prevents intelligible observation by the uninitiated observer. These observational difficulties led to the expedient of killing mating couples in boiling water. The procedure resulted in the first mating-anatomies and inaugurated the studies and use of the technique.[6] Subsequently, the mating-anatomies of other species of *Mesodon* as well as *Stenotrema*, *Allogona*, *Triodopsis* and non-polygyrid snails have been examined.

From the accumulated data, the mating-anatomy technique is of obvious utility in facilitating the detailed examination of the internal (functionally external) anatomy of the penis; in revealing the relationships between parts of the sex-organs; and in aiding the discernment of adventitious folds from structural essentialities in the retracted anatomy. Indeed, some of the structural details so revealed have been utilized for the erection of new groups (Webb, in press).

Furthermore, the morphological-physiological information so obtained aids in the investigation of the genetic-evolutionary factors which have effected the differentiation of these snails.

For example, among the Polygyrinae studied, the mechanics of semen-transfer is a much more precariously adjusted process than in the allied subfamily; and the successful completion of the sex-act depends upon factors of behavior,[7] inter-sex-organ spatial relationships and

[5] Eversion is used here to mean the out-rolling of an organ so that it is turned inside-out during extrusion. An organ may be protruded as the result of the eversion of the penis-sac to which it is attached (*i.e.*, the stimulator of *Deroceras agreste* (L.)).

[6] A prior use of mating-anatomies has been developed by European students.

[7] Species which do not employ biting as a sex-stimulus seemingly can not mate with kinds which do.

the rate-and-type of eversion-entwistment.[8] Also, such exactations are coexistent with structures functioning in the forming and the securement of the spermatophore.[9] The spermatophore-moulding, apical chalice of the penis of *Mesodon thyroidus* (Say) and *M. clausus* (Say), and associated prominent series of ridges—to which spermatophores have been found attached—are good examples of these structures. Evidence that occasionally some of these factors are effective apomixic[10] forces is shown by observations wherein the mating has been noted to be obstructed by out-of-timing malfunctioning. In one case, persistently-faster-eversion prevented a common entwistment of the penis with that of the prospective mate.

The triodopsin species studied, in contrast, are relatively free of such apomixic factors. The less precarious semen-transfer process in this case seems to depend on the greater mechanical stability afforded by the true copulation process (with the insertion of the penis into a vaginoid organ) characterizing these kinds. Not only may non-reciprocal coitus occur,[11] but also cross-copulations between diverse species with such efficiency as to complete the act by at least one[12] of the participants. I have never as yet, however, observed attempts at self-coitus, non-reciprocal mating or interspecies semen-transfer by any of the polygyrin species studied.

Considering these data, the apparent greater divergence among at least the east-of-the-Mississippi Polygyrinae, as contrasted to the Triodopsinae of the same

[8] Penis-entwistment is non-occurrent in some *Stenotrema*.

[9] During mating no spermatophore but merely a common semen-mass forms on the penises in some species.

[10] Apomixy: (adj. apomixic or apomictic): "The phenomenon of limited or no cross reproduction" from Cain (1944), p. 476.

[11] Adventitiously in some species, normally in *Triodopsis fosteri* (F. C. Baker).

[12] *Triodopsis tridentata* (Say) × *T. vannostrandi* (Bland); *T. t. juxtidens* (Pilsbry) × *T. hopetonensis* form *charlestonensis** Mazyck; and *T. tridentata* × *T. hopetonensis** (Shuttleworth). The species with the larger penis or smaller vaginoid organ may be occluded from reciprocation. (* I am indebted to M. H. Sass and Anne Gray Hackney for specimens.)

area, may be due to differences in the two groups susceptibility to the formation of sex-organ-difference barriers to cross-mating within diverging strains. Hence apomixic factors, in addition to the usual isolation-forces, may be, and have been, active agents in promoting the evolution of these Polygyrinae.[13]

Validity of Mating–Anatomy Data

In evaluating the validity of mating-anatomy-derived data, the possibility of technique-induced abnormalities was considered. This question was studied by two procedures (using the larger species of *Mesodon* and *Triodopsis*): (1) the results from mating-anatomy dissections were compared with the results obtained from observing the organs of living animals; and (2) the retracted anatomies were artificially everted to stimulate mating-anatomies.

In obtaining the data from living animals, the turgesced organs of artificially disengaged mating-couples were compared with figures and preserved specimens of the mating-anatomies. Frequently the organs of one of a formerly engaged couple were observed in life, and the other specimen (by being plunged into boiling water) yielded a mating-anatomy. Although de-turgescence and retraction starts immediately, repetitions of the process permitted an adequate study of the organs for the detection of induced abnormality. Either set of data alone would have been untrustworthy; especially observations from the living animals, because the stage of eversion could not have been ascertained without the dissection of mating-anatomies. The two sources of data, consequently, are complementary. I have not been able to make similar comparisons profitably in the smaller species. However, there seems no reason for believing that significantly greater abnormalization occurs with them.

The artificial-eversion technique (subsequently de-

[13] Like phenomena may occur in the rest of the distribution area, but adequate mating-anatomy data are yet lacking.

scribed) is useful in checking the reliability of mating-anatomies—especially in showing the physiology of eversion—and in aiding in the interpretation of eversion-stages.

The results of these evaluations show that mating-anatomies are sufficiently free of technique-induced deformations to constitute a valid source of anatomical data in landsnail studies. In some cases, seeming deformities proved to be apparencies resulting from the comparison of unlike stages of eversion. Snails exhibiting distinct eversion-phases, as *Deroceras,* and occasionally *Allogona* and *Polygyra,* normally exhibit such anatomical variants. Lone mating-anatomies, for these reasons, are almost impossible to evaluate as to normality, and are difficult to interpret until a series accumulates. Generally the larger the available series, the more accurate and reliable are the results. The study of an adequately large series, however, yields data of as great accuracy as that of the better-known techniques.

The most serious deformities yet found in mating-anatomies (in my experience) are those caused by apertural dentition-and-constrictures such as occur in many *Stenotrema* and *Polygyra.* Abnormalizations of this kind result from the retraction of the animal's body with the ensuing wedgement of the sex-organs more-or-less completely into the aperture. When this occurs, contacts between the organs and the hard shell may create various distortions, folds and indentures in the soft, yielding tissues. The subsequent tissue-fixation transforms these blemishes into permanent deformations of the sex-organs.

Deformations of this nature are frequently discoverable by careful dissection, and should be anticipated whenever the sex-organs are found in close contact with the aperture-region of the shell. Deformities are also to be detected by the meticulous comparing of members of a series of mating-anatomies; and the occurrence of sporadic variations may be hypothesized as being deformation-effects. When deformation seems to be occurring,

variation in the method of anatomy—procurement should be tried. Use particularly should be made of crush-or-forceps disablement procedures. Both of these methods overcome dentition-caused deformities, but in turn may possibly cause super-turgescence. As yet, however, I have not detected super-turgor effects in any mating-anatomy so obtained.

Techniques

As mentioned above, the technique is mainly that of killing mating-couples while the sex-organs are extended. Because the animals must be disabled almost instantly from retracting or deforming the sex-organs in response to disturbance, boiling water seems to be the most suitable fixative. The proximity of specimens and killing-water is also important; since occasionally (in some species) the organs must be fixed within seconds following their eversion. A small, portable water-heater is of use in this regard. At times pouring boiling water on the mating pair is expeditious; under group confinement methods, the mating-couples usually can be successfully transferred to the water-container. In dealing with slugs (such as *Deroceras*) the mating at times occurs on the soil in the cage. The ensuing difficulty in quickly transferring such more-or-less slimy pairs can be met by inserting a spatula below the courting animals, and then transferring the earth-and-slugs into boiling water at the instant of penis-entwistment. The efficiency of the fixation-process is very important; because, the sooner the tissues are fixed, the less is the likelihood of retractive-processes deforming the extruded organs. In working with the smaller species, it is advantageous to destroy the aperture or to crush the shell as soon as the specimen is withdrawn from the boiling water. Otherwise the fixed body-and-organs probably will be sucked into the shell (from vacuum-effects accompanying cooling) and may be damaged. Such precautions are less necessary in dealing with triodopsin species, as the sex-organs are more

compact and the coition process frequently effectively anchors the organs against retraction. Except for the immediate initiation of extractive procedures, the larger polygyrids seldom require the destruction of the aperture to overcome vacuum-effects.

Another technique is that of crush-disabling mating specimens. By crushing the shell, the sex-organ retractors are more or less completely disabled, and the collector may then place the animals in a preservative with a likelihood of having the organs fixed in the extruded condition. I have used this method as a field expedient with *Helicella caperata* (Montagu) in England, when numbers of mating-specimens were found along a roadway after an autumn rain. The procedure is also of laboratory value in preventing aperture-caused deformations to the mating-anatomy such as occasionally occur in some species as *Stenotrema* and *Polygyra*. Care of course should be taken to avoid crushing the extruded organs when crushing the shell.

Forceps-disabling of mating couples is a modification of the preceding. The method consists of seizing the extended mating-animal between the shell and extruded organs with strong forceps. The animal is then killed by immersing the snail-containing-forceps-tips in boiling water. The objective is to inhibit all apertureward retraction until fixation immobilizes the extended body and organs.

Lastly, there remains the artificial-eversion technique. The method is a useful adjunct to the study of mating-anatomies, and is of special use in permitting evaluation of the sex-organ structure of species of which mating-anatomy material is lacking. The method consists essentially of turning the retracted penis (as applied to polygyrids) inside-out. In successfully artificially-everted anatomies, the organs exhibit only the essential form as seen in mating-anatomies. Chance configurations are still present to some degree due to the untumefied condition of the tissues. The artificial-eversion technique may

be a useful expedient for institutional workers where preserved material constitutes the bulk of the available specimens. Unfortunately, the method is generally unsuccessful when applied to the smaller species, and a foreknowledge of the mating-anatomy of an allied species is desirable for the most accurate evaluation of results. Artificial-eversion may be accomplished by the following procedures.

The penis-sac may be slit longitudinally from the atrium apicalwards. The penis-tip can then be forced downwards through itself. Freshly drowned material is preferable, and occasionally may be everted without slitting by merely turning-back the atrial end of the penis and forcing the remainder through it. The longitudinal slit is used to reduce internal friction, and can be extended to near the penis-tip. In such case, the tip needs only to be evaginated a short distance, and not the entire length of the penis. Only blunt forceps or other instruments should be applied to the penis-tip as its rupture impairs further progress and may so abnormalize the anatomy as to render it useless for study. It is also frequently possible to slit the penis almost to the tip, and then to insert one point of a pair of forceps into the penis-tip cavity, and to allow the other to rest externally on the tip. By a prying motion, the outer penis-tip can then be forced inwards and the penis-wall simultaneously stretched outward. The effect of these combined pressures is the eversion of the penis-tip. The slit remainder of the penis then automatically becomes reorientated in the everted position. A frequent difficulty in such manipulations is to have the zone of slitting mutilate essential structures. This must be overcome by experience. Satisfactory results usually are not attained until several anatomies of any given species have been everted.

In occasional triodopsin species, the penis can be everted solely by pressure. In species where non-penial parts of the sex-organs are also evertable, it may be possible to evert these artificially. I have as yet utilized

the method solely for penis-eversion in *Mesodon, Triodopsis* and *Limax;* and for atrium, penis and female-organ eversion in *Arion.*

Culture Methods for Procuring Matings

Aside from the procedures used for obtaining mating-anatomies, the remaining problem is that of how to obtain enough matings to yield adequate numbers of anatomies. The research-time available to the investigator and the species studied will be factors in determining the procedures adopted. Two methods seem basic: (1) the group-culture method with the maintenance[14] of numerous individuals of a species in a common container; or (2) the species-isolation method with the maintainence of apomixic aggregations of individuals.

The species-isolation method frees the investigator from the assiduous surveillance associated with group culture, permits planned matings (which facilitate photography), and minimizes the occurrence of emergency anatomy-fixation preparations because a long-hoped-for-mating is inopportunely in progress. The isolation method is subject to the criticism of somewhat abnormalizing mating procedures.

The group-culture method is advantageous in offering more natural living conditions such that the sex-act is not effected by possible enhancements of the sex-urge from unnatural restraints. The investigator, however, is disadvantaged in having less of an opportunity of witnessing the onset of courtship than results from the controlled alternative procedure. Species which engage in regular coitus may be exploited more profitably by the group method than those which engage in the fleeting sperm-transfer process.

A very satisfactory culture method results from combining the preceding by the periodic grouping of isolation-cultured specimens in a common mating-cage for several days. The specimens are then kept under as

[14] = Maintenance.

constant observation as possible. Several such cages may not contribute to the conventional appearance of a desk, but may result in numbers of valuable mating-anatomies; for dinner-table operations, coffee may prove of value as a fixative!

Food and Caging

Adjunctive to methods for obtaining mating-anatomies, maintainence factors are an integral part of any mating-anatomy technique. The following procedures may be of use to other investigators, and are cited not as an exposition of the best procedures to be used, but rather as successful ones. Little, however, is added to the already existent general information on the subject.

In captivity most polygyrids seem omnivorous but adverse to feeding on fresh, green vegetation. Early in the studies, the specimens were periodically fed oatmeal (rolled oats), bread, fresh lettuce, carrot, sweet-potato, and irish-potato. It was observed that eventually some specimens ceased to feed on anything except oatmeal. The expedient was then adopted of preparing oatmeal as a powder and mixing it with similarly prepared dried grass-cuttings. The objective was to prevent selectivity by forcing the specimens to feed on the complete mixture and so benefit from the more natural diet. This has become standard procedure and stock bottles of powdered oatmeal, babyfoods, cereals, egg-shell, green grass-cuttings, brown tree leaves (maple, mulberry and elm[15]), dandelions and nettles are used in preparing various mixtures. Protein-rich dried commercial fishfoods (dried salmon-eggs, shrimp and clam-meal) are occasional additions to the mixtures. Carrot, lettuce and sweet-potato are also fed raw as available. During feeding, the powdered-food mixtures are placed in the cages on small squares of paper. This expedites the removal of molding, uneaten food.

[15] *Acer saccharinum* L., *Morus alba* L. and *Ulmus* sp., (See "Gray's New Manual of Botany, etc.," American Book Company, N. Y.).

For cages, I have found cans (two to six inches in diameter) and heavy paper-boxes to be most suitable. A common difficulty in maintaining caged landsnails is the presence of dry corners and areas which act as aestivation-traps by forcing roaming specimens into aestivation before they happen to re-enter regions of activity-maintaining humidity. The resultant too-frequent aestivations interrupt growth and reproduction of the specimens. Aestivation-traps sometimes can be eliminated by judiciously regulating cage soil-depth. An increase in soil-depth usually increases cage-humidity; a decrease in soil-depth decreases humidity.

Can-cages are especially useful in isolation culturing, being of minimum size, easily cleaned and requiring only the addition of a cover-glass and a quantity of soil. Ventilation is increased after punching holes in the can, or after hammering slight depressions in the upper edge where the can is overlain by the cover-glass. The resultant orifices should be much smaller than the size of any of the inmates, especially in dealing with slugs. However, the proper ventilation of can-cages is difficult—humidity tending to be excessive. For species intolerant of much or prolonged periods of moisture, paper-cups are usable in place of cans, and sometimes give better results.

Earth-filled, glass-covered paper boxes were used in place of the conventional glass-and-metal terraria, being advantageous in overcoming the excessive humidity commonly present in the latter. Furthermore, cage-porosity allows habitat-like-moisture-cycles to be created artificially. The only instances I have had of *Helminthoglypta*[16] thriving in captivity were when they were maintained in such heavy paper-box cages.

Summary

(Mollusca, Gastropoda, Pulmonata, Polygyridae, with brief, casual mention also of *Arion, Deroceras, Helicella, Helminthoglypta,* and *Limax.*)

[16] *Helminthoglypta umbilicata* (Pilsbry) and *H. dupetithouarsi* (Deshayes).

(1) Studies of the everted (functioning) sex-organs are useful in showing the relations between parts and in aiding accurate observation of the structure and internals of the retracted organs.

(2) Quickly killed mating-couples exhibit the organs in the everted condition suitable for study—such cadavers are termed "mating-anatomies."

(3) Boiling water is useful as a fixative and killing agent.

(4) Crush-and-forceps disabling techniques are useful in obtaining more perfect mating-anatomies.

(5) Artificial-eversion is an adjunctive technique useful in the study of mating-anatomies and the physiology of eversion.

(6) Group and isolation culture methods are useful basic laboratory procedures in procuring matings.

(7) The method found most useful in obtaining matings is that of periodically grouping isolation-cultured specimens.

(8) Aperture-caused deformities are the only noteworthy deformation of the sex-organs found to result from the mating-anatomy technique; such deformation can be prevented.

(9) Mating-anatomy studies yield data of use in evaluating the genetic-evolutionary forces affecting polygyrids. The methods are also applicable to other groups of gastropods.

(10) Semen-transfer in many Polygyrinae is by an entwistment of penises with an exchange of seminal matter—the process is mechanically unstable and favors apomixy.

(11) Semen-transfer in many Triodopsinae is by the insertion of the penis into a vaginoid organ—the process is mechanically efficient and interspecies cross-copulation has been noted.

(12) East of the Mississippi Polygyrinae seem more susceptible to apomixic factors than the coexistent Triodopsinae.

(13) Paper boxes are useful in overcoming excessive cage-humidity.

(14) Cans make useful cages for isolation-culture procedures.

(15) Powdered foods can be successfully used and are an aid in obtaining natural diets.

LITERATURE CITED

(The pagination of especially pertinent data is cited in parentheses).

Archer, A. F.
1933. *Univ. of Mich. Occasional Papers Mus. of Zool.*, No. 276, pp. 1–8 (p. 6–7).

Binney, W. G.
1878. *Harvard Coll., Bull. Mus. Comp. Zool.*, Vol. 5.

Cain, S. A.
1944. "Foundations of Plant Geography." Harper and Bros., New York, pp. 3–556 (p. 476).

Gerhardt, Ulrich
1933. Berlin, *Zeitschrift für Wissenschaftliche Biologie*, Abteilung A, *Zeitschrift für Morphologie und Ökologie der Tiere.* Band 27. Figs. 1–11, pp. 401–450. [Library of Cong. microfilm.]

Howard, Roscoe C.
1942. Thesis Abstract, *Biol. Abstracts* (1945) 19(10) 23218—W. M. Bowden.

Jones, D. T.
1940. *Univ. of Utah Bull.*, Vol. 31, No. 4, pp. 1–43.

Pilsbry, H. A.
1905. *Acad. Nat. Sci. of Philadelphia, Proc.*, pp. 211–290, Pl. 11–27. (p. 249–250, Pl. XXI, Fig. 25).
1909. *Ibid.*, pp. 495–516, Pl. 19–21, (Pl. XX, Fig. 3, 4.)
1930. *Ibid.*, pp. 303–327, Pl. 23–27.
1940. *Ibid.*, Monogr. 3, Vol. 1, Part 2, pp. 575–994, Figs. 378–580, (p. 915, Fig. 524, 2a).

Simpson, G. B.
1901. *Univ. of State of N. Y. State Mus., Bull.* No. 40, Vol. 8, pp. 241–311, Pl. 1–28 (Pl. 3, fig. 3). [From Libr. of Cong. microfilm.]

Webb, G. R.
1943. *Amer. Midland Nat.*, Vol. 30, No. 2, Reprint, pp. 341–345, Figs. 1–5 (p. 344).
In press. "Comparative Observations on the Mating of Certain Triodopsinae" Univ. of Pennsylvania, Zool. Lab., Philadelphia, *The Nautilus.*
In press. "The Sex-organs and Taxonomy of certain Polygyridae of the genus *Mesodon.*" *Ibid.*

SHORTER ARTICLES AND DISCUSSION

INVERSION GRADIENTS AND SELECTION IN ECOLOGICAL RACES OF *DROSOPHILA FUNEBRIS*

DUBININ and Tiniakov (1945, 1946a) have shown that the species *Drosophila funebris* contains ecological races which differ in the relative frequencies of inversions in some of their chromosomes. Thus, the populations of this species which inhabit urban centers (Moscow, Ivanovo) contain relatively more inversion heterozygotes than populations of rural localities. The frequencies of the inversion heterozygotes in the populations of at least some localities do not remain constant in time but undergo changes from month to month; these changes are evidently connected with seasonal changes in the environment in which the flies live, and represent an adaptive response controlled by natural selection. In our experiments on natural selection for populational inversions (Dubinin and Tiniakov, 1946b) we found that carriers of different gene arrangements react differently to changes of temperature. For example, some chromosomal types survive hibernation more easily than others.

The above findings raise a number of new problems. It may, for example, be asked whether the urban and the rural ecological races of *Drosophila funebris* are discrete, or connected by transitional populations which inhabit the suburban localities and other territories of a character intermediate between the extremely urban and the purely rural types. The problem of whether or not there is a dependence between the distribution of inversions on one hand and the degree of industrialization of different cities and towns on the other may next be considered. Finally, the response of the various inversions to changes in the general climatic factors may be examined. Data bearing on these problems are reported in the present article.

Studies made in the spring and summer of 1945 have shown that the populations which inhabit the central portion of the city of Moscow, and which contain as many as 88.1 per cent. of inversion heterozygotes, are connected by transitional populations with the populations of suburban villages having about 1.8 per cent. of inversion heterozygotes, and finally with the populations of more remote northern villages which seem to be free of inversions.

The city of Moscow may be divided into zones inhabited by populations of *Drosophila funebris* which contain various frequencies of inversion heterozygotes. In the central portion of the city (Mokhovaya, Arbat, Samoteka) 88.1 per cent. of the flies are inversion heterozygotes. Around this there is a zone (the so-called "Circle B," Meshchanskaya, Voronzovo Pole) in which as many as 55.5 per cent. of the inhabitants are inversion heterozygotes. The third zone surrounds Moscow in the region of the Circuit Railway (Hippodrome, Petrovsko-Rasumovskoe, Serebriany Bor, Vorbiovy Gory, Ismailovo). This zone may be regarded as transitional between the urban and the rural types of environment. Here the frequency of inversion heterozygotes amounts to only 12.1 per cent.

The fourth zone includes suburban villages which are from 20 to 200 kilometers distant from Moscow. Here the heterozygote frequency is only 1.8 per cent. The fifth zone are villages located from 200 to 500 kilometers north of Moscow. In this fifth zone no inversions have been found.

The frequencies of inversion heterozygotes in different populations within any one zone vary within relatively narrow limits. Thus, five populations have been studied in the third zone, that of the Circuit Railway, and the following frequencies of inversion heterozygotes have been found in these populations: 16, 10, 9, 12 and 12 per cent., respectively. There is no doubt that the frequencies of inversion heterozygotes form a remarkably regular gradient, with a maximum in the central part of the city of Moscow and a minimum in the remote northern villages.

We have shown (Dubinin and Tiniakov, 1946b) that in a given environment the concentration of inversions tends toward a certain optimal value. The concentrations of inversions are higher in some and lower in other environments. The differences between the environments in the center of the city of Moscow and in rural districts far from the city are obviously very great, and it is also obvious that the suburban localities have by and large transitional environments. The different concentrations of the inversions in the five zones examined appear, then, to reflect the differences in the environments in which the flies of these zones live.

It must, however, be pointed out that the migration pressure may also give rise to a gradient between the zones with the high and the low concentrations of inversion heterozygotes. Indeed,

the city population is a high and precipitous peak located in the midst of a great expanse of territories occupied by the populations of rural areas. Interchange of migrants between the city and the country populations might produce a gradient between these populations. Such migration undoubtedly takes place. Apart from the active movements (flight) of the flies, they are transported inadvertently by man with vegetables and other products. Such transport by man occurs mainly in one direction, namely, from the country to the city. The populations that live at the extreme ends of the gradient, namely, the population of the center of Moscow on one hand and the rural populations on the other, are made different by the action of natural selection. The intermediate zone, that of the Circuit Railway, is certainly characterized by ecological conditions which combine the characteristically urban and rural elements. Natural selection would, then, be expected to create in this intermediate zone some transitional populations. However, the effects of migration from the rural populations in the direction of the city should be felt most clearly also in this intermediate zone. It is difficult at present to tell the relative importance of natural selection and of migration pressure in the determination of the characteristics of the populations of the Circuit Railway zone.

In the Moscow region, populations of *Drosophila funebris* contain several inversions. The five most important ones among them are as follows: Inversion II–1 is the most frequent one in the urban as well as in rural localities. The frequencies of this inversion are strikingly different in the city and in the country: its concentration in the center of the city is 55.4 per cent. and in the rural zone 0.9 per cent. Inversion II–2 is the second most frequent one. Its frequency in the center of the city is 19.2 per cent., and in the rural areas 0.15 per cent. Inversion IV–1 is the third in frequency; its concentration in the center of the city is 11.5 per cent., and in the rural areas 0.05 per cent. Inversion III–1 is found only in the city, and its frequency is low (0.48 per cent.). Finally, inversion II–3 is found in the city as well as in the rural zones, its frequency being about equally low in both (0.45 per cent.). It seems, then, that each inversion has its own ecological optimum; different inversions respond differently to various environmental conditions. Thus, inversion II–1 is 61.5 times more frequent in the urban than in the rural areas, inversion II–2 is 128 times, and inversion IV–1 230 times more frequent in the city than in the country.

These data show a sharp difference in the composition of the populations of an industrialized city and of rural areas. Although three zones of diminishing inversion frequencies can be distinguished in the city of Moscow, it is strikingly apparent that within only a few kilometers from the zone of the Circuit Railway one invariably finds populations with a decided predominance of the standard gene arrangements in all chromosomes (*i.e.*, free of inversions). The same kind of sharp difference during transition from an urban to a rural locality has been observed also in and near the cities of Saratov and Erivan.

Comparison of the frequencies of inversion heterozygotes in different cities also shows the importance of ecological adaptability for the determination of the caryotypic characteristics of populations. We have examined the populations of seven cities and towns located far apart from each other, some of them in the northern and others in the southern parts of our country. The concentrations of the inversions proved to be different in different cities. The highest frequency of inversion heterozygotes has been found in Moscow (88.1 per cent. in the center, 55.5 per cent. in the zone of "Circle B," see above). The next highest frequency of inversion heterozygotes has been encountered in the city of Saratov, namely, 53.9 per cent. In the city of Ivanovo the frequency of heterozygotes is also high, 37.2 per cent., but it is lower than those in Moscow and in Saratov. In Erivan (the data of E. Pogossian) inversion heterozygotes are less frequent—21.2 per cent. In the towns of Noginsk and Michurinsk inversion heterozygotes are about as frequent as they are in the Circuit Railway zone of Moscow, namely, 12.1 per cent. and 8.7 per cent., respectively. The samples in Voronezh were taken after this city had been destroyed by the Germans; it may be significant that the frequency of inversion heterozygotes proved to be that characteristic for rural localities (0.9 per cent.). A low frequency of inversion heterozygotes is recorded also in the city of Alma-Ata (1.48 per cent.), but this city, in Turkestan, is a special case, since it is one of the garden-like cities not uncommon in these parts.

A correlation between the degree of industrialization of an urban center and the frequency of heterozygotes in its population seems to be clearly indicated. The highly industrial city of Moscow has the highest concentration of inversions, and that of the garden-like Alma-Ata the lowest (Voronezh is, as indicated above, an exception which may easily be explained).

An analysis of the distribution of different inversions in the cities examined reveals certain regularities concerning the influence of the general climatic condition on the natural selection of inversion heterozygotes. Starting with the city of Ivanovo in the North, and down to Erivan in the South, there is observed a climatic gradient of the frequencies of the inversion II–1. At Ivanovo, the II–1 inversion heterozygotes make up 80 per cent. of all inversion heterozygotes found. At Moscow, these inversion heterozygotes amount to 72 per cent. of the total, at Michurinsk 54 per cent., Saratov 35 per cent., and finally at Erivan 1.3 per cent. The inversion II–2 shows a relation to climate opposite of that exhibited by II–1. Namely, the inversion II–1 heterozygotes amount to 3 per cent. of all heterozygotes at Ivanovo, 15 per cent. in Moscow, 45 per cent. in Michurinsk, 46 per cent. in Saratov, and 95 per cent. in Erivan. Inversions II–1 and II–2 can not occur together in the same chromosome, but the frequencies of neither inversion are high enough to cause an appreciable elimination of one by the other. The gradients in the frequencies of both inversions are caused by natural selection.

The climatic gradients are manifested independently from the frequencies of inversions in the populations of cities and towns. The gradient of the degree of industrialization of the cities studied does not coincide with the climatic gradient. For example, in the three zones of the city of Moscow (see above), the overall frequencies of inversion heterozygotes are quite different, namely 88, 55 and 12 per cent. And yet, the climatic characteristics of these zones are identical, as shown by the fact that the heterozygotes for the inversion II–1 make up in all zones about 72 per cent. of all heterozygotes. The degree of saturation of a population with inversions is a function of the degree of industrialization, but the qualitative character of the chromosomal variability is determined by climatic factors. In the North the populations of industrial localities show high frequencies of inversion II–1, while in the South inversion II–2 gains the upper hand. Inversion IV–1 seems not to vary with the climate.

Summary

The populations of *Drosophila funebris* which inhabit the central part of the city of Moscow contain 88.1 per cent. of inversion heterozygotes; the populations of the less centrally located parts of the city 55.5 per cent., those of the margins of the city 12.1

per cent., of the suburban villages 1.8 per cent., while in the country from 200 to 500 kilometers north of Moscow no inversion heterozygotes at all have been found. High concentrations of inversion heterozygotes have been observed in other cities as well (Saratov, 53.9 per cent., Ivanovo 37.2 per cent., Erivan 21.2 per cent.), while in small towns, in a city exceptionally rich in gardens (Alma Ata), and in a city devastated by the war (Voronezh) the concentrations of inversion heterozygotes are low. While the overall frequencies of inversion heterozygotes are, thus, connected with the degree of industrialization of the locality, some inversions are relatively more frequent in the North while others are relatively more common in the South. These qualitative and quantitative variations in the concentrations of inversion heterozygotes in different populations represent adaptive responses induced by the action of natural selection.

N. P. Dubinin
G. G. Tiniakov

Institute of Cytology, Academy of Sciences
Moscow, U.S.S.R.

LITERATURE CITED

Dubinin, N. P., and G. G. Tiniakov
1945. Am. Nat., 79: 570–572.
1946a. Am. Nat., 80: 393–396.
1946b. *Jour. Heredity*, 37: 39–44.

STABILITY OF FAMILY NAMES: SOME PRINCIPLES AND PROBLEMS[1]

Family names are among the most widely used of all the technical names for animals or groups of animals. Introductory text-books are commonly organized on that basis, beginning students learn to classify the subjects of their study by them, and specialists in non-taxonomic fields may recognize few names beyond those of families.

Nowhere is the above more true than in entomology, because of the vast numbers of genera and species involved. Whereas the ornithologist, for example, can recognize and give both generic and specific, and perhaps even subspecific, names for the birds in his region, the taxonomic entomologist often does well to know a reasonable proportion of the family names of the insects of his

[1] For brevity, only family names are referred to, but the discussion will of course apply as well to the names of subfamilies.

area, outside of the common genera and species and those of his own particular speciality. The economic entomologist will recognize species of economic importance, but beyond that will rely mainly on group names for the various forms that he meets.

Many family names, particularly in entomology, have become quite generally used and understood as common names, not only by taxonomists, but also by biologists, general zoologists, science teachers, etc. Such names as tabanids, tipulids and sarcophagids have become nearly as well known as their counterparts of horseflies, crane flies and flesh flies. In some cases, such as the parasitic flies of the family Tachinidae, there exists no common name for the group other than that founded upon the family name itself. In other cases, it is convenient to use a common name based on the family name, such as the noctuid moths or Noctuidae (cutworms, stalk borers, earworms, etc., now known as the Phalaenidae), because of the diversity of common names for groups of species within the family, or because the common names apply to larval forms and not to adults.

Obviously, therefore, the changing of family names may have more extended repercussions than the changing of generic or specific names. No matter how vehemently specialists may argue for or against changes in names, they will have to admit that, because of their specialized knowledge and training, no change will be likely to cause them more than temporary annoyance and occasional slips in their bookkeeping. However, to specialists in other fields of biology, to students of other sciences, and to nontechnical workers, the changing of familiar and long-recognized names and the continued use of conflicting names by different specialists contribute not only to confusion but to a low regard in some quarters for both taxonomy and nomenclature. For example, the pictured-winged flies of the family long known as the Trypetidae are commonly called the trypetids, and they are widely known and recognized because of such common species as the cherry fruitflies, the apple maggot or "railroad worm," the Mediterranean fruitfly, the round golden rod gall maker and many others. Yet the appearance of such names for the family as Trupaneidae, Trypaneidae, Euribiidae and Tephritidae leaves the average reader only bewildered.

Some changes are of course inevitable, such as the necessity of renaming homonyms in order to prevent the confusion of having identical names in use for two different groups. In many cases,

however, we need prompt action by the International Commission on Zoological Nomenclature in order to clarify certain general principles and to bring system into the often confusing terminology of family names.

Articles 4 and 5 of the International Rules of Zoological Nomenclature, simple and direct as they seem, have been the subject of considerable dispute and conflicting interpretations. Some of the doubts about family names have been resolved by Opinions 133 and 141, published in 1936 and 1943 by the International Commission. However, in spite of the clear-cut statement of principles contained in Opinion 141, there still remain several points of contention. Even though in some cases the answer may seem obvious, or the probable answer is indicated by the discussion in the above or other Opinions, nevertheless a more comprehensive statement of principles by the International Commission would clear up certain difficulties that now exist in the nomenclature of family names, as indicated in the following discussions.

(1) *Shall a family name, based on an included generic name, have priority and authorship from the date of its first proposal for a super-generic group, whether originally called a family or stirps or etc., and regardless of the patronymic ending employed?*

The use of "-idae" as a uniform patronymic ending for family names was first advocated by William Kirby in 1813 (*Trans. Linn. Soc. London*, 11: 88, footnote). Although the idea soon found favor in many quarters, it was naturally many years before it was consistently and universally applied. Meanwhile, authors used such terminations as "-ites," "-ides" and "-ina." In many cases, these were called "family" or "subfamily"; in others, "stirps," "section," "natural order," etc.

Many of our best-known family names date from this period when there was no uniform family patronymic. The critical study of the origins of these names will sometimes reveal that insistence upon the "-idae" ending for determining priority would overthrow long-established names in favor of unknown or obscure ones. In the two-winged flies (Class Insecta, Order Diptera), for example, a number of our familiar family names were apparently first used by Edward Newman in 1834 ("Attempted Division of British Insects into Natural Orders," *Ent. Mag.*, 2: 379–431), who consistently used the suffix "-ites" to designate his "natural orders" (= modern families) in such combinations as Bibionites, Psychodites and Chironomites.

In fairness to early taxonomists, who based their super-generic names on included genera, but merely with different endings to designate the groups, it is believed that the question should be answered in the affirmative.

A suggestion to this effect, in the nature of a proposed amendment to the International Rules, was also submitted to the commission some years ago (*cf. Science*, 68: 102–104, 1928; *Ent. News*, 40: 332, 1929), as was the principle discussed in the third section.

(2) *When a family name is changed because the name of the type genus is found to be a homonym, must the new family name be founded upon a new generic name replacing the preoccupied one or upon the next available generic name?*

Although it is believed that the answer will favor the first alternative, it would be well to have a clear and unequivocal statement. The continuity of the type concept is important, when a certain generic group is used as the standard of reference for a family. If a preoccupied generic name which is a familiotype (type genus of a family) should be replaced by a name which is not isogenotypic with it, then the new family name might conceivably be based on a group of species which might some time be recognized as quite different from the original type genus. We would then find that the family name would pass to the new segregate, and we would be forced again to rename the original and long-established genus and the family name founded upon it.

To cite a definite example, a large and common family of pictured-winged flies, similar to the trypetid fruit-flies in appearance and size, has been well known for years under the name Ortalidae. Not until 1932 was it pointed out that the generic name *Ortalis* Fallén (1810) is preoccupied by *Ortalis* Merren (1786) in ornithology. Two authors almost simultaneously proposed new names for the family, Otitidae and Platystomidae.[2] In each case, the name is based on an entirely *different* genus than the original Ortalidae, thus changing the family's type standard. The choice of Platystomidae was particularly unfortunate, since it is based on a genus in another subfamily from the original *Ortalis*, and one which is recognized by some authors as a family in its own right.

(3) *Must a family name be changed when its type genus is found to be a synonym?*

[2] Based on *Otites* Latreille, 1804, and *Platystoma* Meigen, 1803.

There is no question that, in order to avoid possible confusion, a family name must be changed when the name of the type genus upon which it is based is found to be a homonym.

Changing with synonymy is not so convincing. A homonym is forever dead: it was stillborn and can not be revived; but it is always possible to resurrect and reinstate a synonym. A homonym engenders confusion because it has the same name as some other animal group; a synonym is a distinctive name in itself, but merely superseded by an older name, sometimes only as a matter of opinion. A synonym is always present in the literature on the family, and a family name based on it would always represent the same type concept, regardless of any changes in the prevailing opinion of its status and synonymy. It is not possible to foresee the trends of future revisionary work and the results of the discovery of thousands of new species. Genera may be combined, later separated, perhaps recombined, with refinements in classification, discovery of annectant species, etc. If a family had to be renamed because of synonymy, there might be frequent changes or vacillation in the family name.

Two examples may be cited by way of illustration:

(a) In the order Diptera (Insecta), the Meigen (1800) generic names (which were first brought to the attention of dipterists by Hendel in 1908) under the Rules supplant the well-known and century-old names of Meigen (1803) for many common genera that gave their name to large and cosmopolitan families. Assuming for present discussion that the 1803 generic names must be changed (which is opposed by some workers), why should we necessarily have to change the familiar Chironomidae to Tendipedidae, or Tachinidae to Larvaevoridae, for example?

(b) The so-called false crane flies were known for years under the family name Rhyphidae, based on *Rhyphus* Latreille (1804). Not until the early twentieth century did authors change to Anisopidae (or Anisopodidae) based on *Anisopus* Meigen (1803) with *Rhyphus* as synonym. Following Hendel (1908), however, some workers changed to Phryneidae, based on *Phryne* Meigen (1800) (= *Anisopus* Meigen, 1803). For the next few decades, dipterists were divided on the use of the 1800 names, and both Phryneidae and Anisopodidae existed in the literature. Finally, as a crowning touch, careful research into the place of the 1800 names in the taxonomic structure (Stone, 1941, *Annals Ent. Soc. Amer.*, 34: 413) revealed that both *Phryne* and *Anisopus* were

synonyms of the still older but hitherto overlooked *Sylvicola* Harris (1776). Thus the family name can once more be changed, to Sylvicolidae, which incidentally is a name once used in ornithology for the American wood warblers. How much senseless change would have been avoided, through the years of changing synonymy, by the continued use of the original name, Rhyphidae!

(4) *Is a family name to be rejected as a homonym of another family name?*

It is possible that each case might have to be considered on its own merits, but the statement of a general principle should help in many situations. Two problems will illustrate different ways in which family homonyms may arise.

(a) In the preceding section, the family of the false crane flies was found to have had four different family names as a result of successive changes in the recognized synonymy of the type genus. Although the name Sylvicolidae has not yet been noticed in the literature, it is the logical step if the nomenclature of family names continues as it has in the past. However, the name Sylvicolidae has already been used in ornithology for the American wood warblers, although later abandoned when its type genus (*Sylvicola* Swainson, 1827) was found to be a homonym. Does this existence of a previous though now unused Sylvicolidae preoccupy the use of the name?

(b) Are identical family names, which are based on similar but not identical (and therefore not strictly homonymous) generic names, to be considered homonyms?

For example, in the "Statement of the Case" in Opinion 153 (p. 199) it was pointed out that a situation appeared to require the use of the name Psilidae for a family of bethylid wasps, based on *Psilus* Jurine (1801). Fortunately, with the suppression of the "Erlangen List," the name *Psilus* ceased to be available, for otherwise we might have had a family Psilidae in the Hymenoptera, based on *Psilus* Jurine (1801), and the identical name Psilidae in the Diptera, based on the valid and distinct name *Psila* Meigen (1803). As a similar situation, there is a family name Laridae both in Aves and in the Insecta (Coleoptera), based respectively on *Larus* Linnaeus and *Lara* Leconte.[3] What shall

[3] I am indebted to Dr. E. Gorton Linsley for calling my attention to the case of Laridae, and also to the fact that family names with a similar sound but different form are Larridae in the Hymenoptera (from *Larra* Fabricius) and Lariidae in Coleoptera (from *Laria* Scopoli). The last two names undoubtedly are sufficiently distinct, for we have the example set by the Inter-

be done with family names based on valid generic names which differ only by ending in -us and -a, where the family names derived from the stem of the name of the type genus are inevitably identical?

(5) *What spelling is to be considered proper and official in the case of certain kinds of family names that have appeared in both short and long forms?*

A few examples from the Diptera will suffice: Acroceridae or Acroceratidae; Empidae or Empididae; Trichoceridae or Trichoceratidae; Dolichopidae or Dolichopodidae.

Inasmuch as Opinion 36 (p. 85) called attention to a change from Dioxoceridae to Diozoceratidae, it is to be presumed that the longer form, based on the full stem of the name of the type genus, is the proper and correct spelling under Article 4 of the Code. It would promote uniformity, however, if the commission would review the interpretation of the stem of the names of type genera where the proper combining form is not apparent in the generic name itself (as in *Acrocera, Empis, Dolichopus, Termes, Leptocerus,* etc.).

A statement of general principles from the International Commission would aid in solving many problems in the nomenclature of family names. Even beyond this, however, it is believed that the preparation of an "Official List of Family Names in Zoology" would do much to promote stability and uniformity in the terminology of these widely used names.

In addition to a request for the statement on general principles, therefore, a proposal has been made that the International Commission on Zoological Nomenclature, with the aid of specialists on the various groups of animals, undertake the preparation of such an "Official List of Family Names in Zoology." When cases are discovered that appear to warrant Suspension of the Rules in order to prevent greater confusion than uniformity, these can then be promptly submitted to the commission for action.

The idea is by no means new, though not previously proposed as an "Official List." At the Fourth International Congress of Entomology (Ithaca, N. Y.) in 1929, in the discussion of a paper by Dr. A. L. Melander, Dr. Karl Jordan expressed the view that

national Commission in Opinion 140, in which official sanction was given to the names Meropidae and Meropeidae, respectively based on *Merops* Linnaeus and *Merope* Newman.

family names should be stabilized by means of a list of "nomina familiarum conservanda" prepared by a committee for each large order.

Undoubtedly, the difficult cases in family names would eventually be discovered and settled in due course. Rather than wait for the haphazard discovery of problems and the slow accumulation of approved names, however, it is believed that more good would result from the active research and preparation of a comprehensive list that would be a contribution to stability and an orderly nomenclature.

CURTIS W. SABROSKY

U. S. PUBLIC HEALTH SERVICE*

* Since this paper was written, the author was released from service and is now employed by the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.